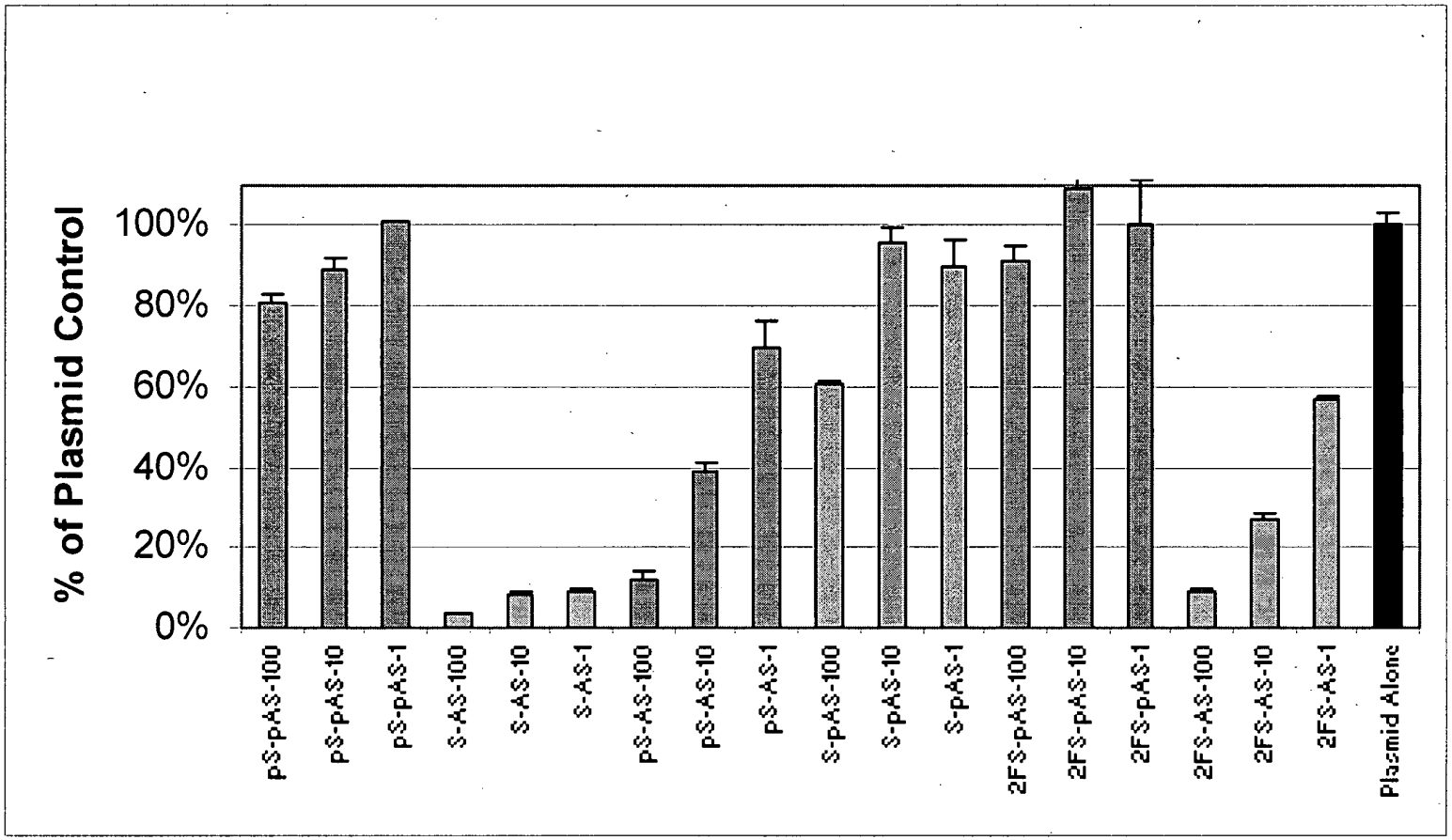


Figure 1A. Introduction of orthoester modifications to the sense strand of siRNA duplex results in a functional entity, 24 hour time point.

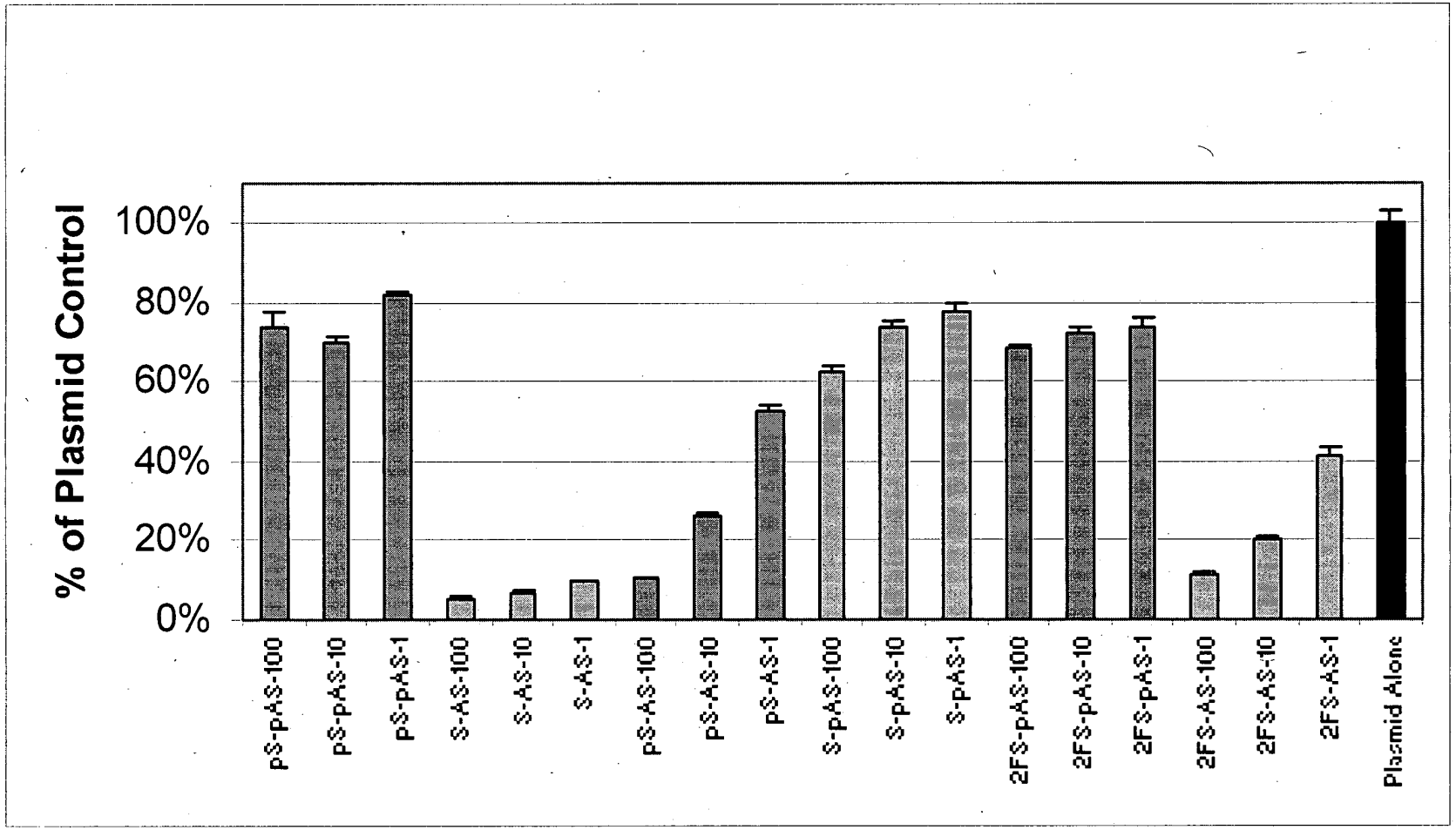


Figure 1B. Introduction of orthoester modifications to the sense strand of an siRNA duplex results in a functional entity, 48 hour time point.

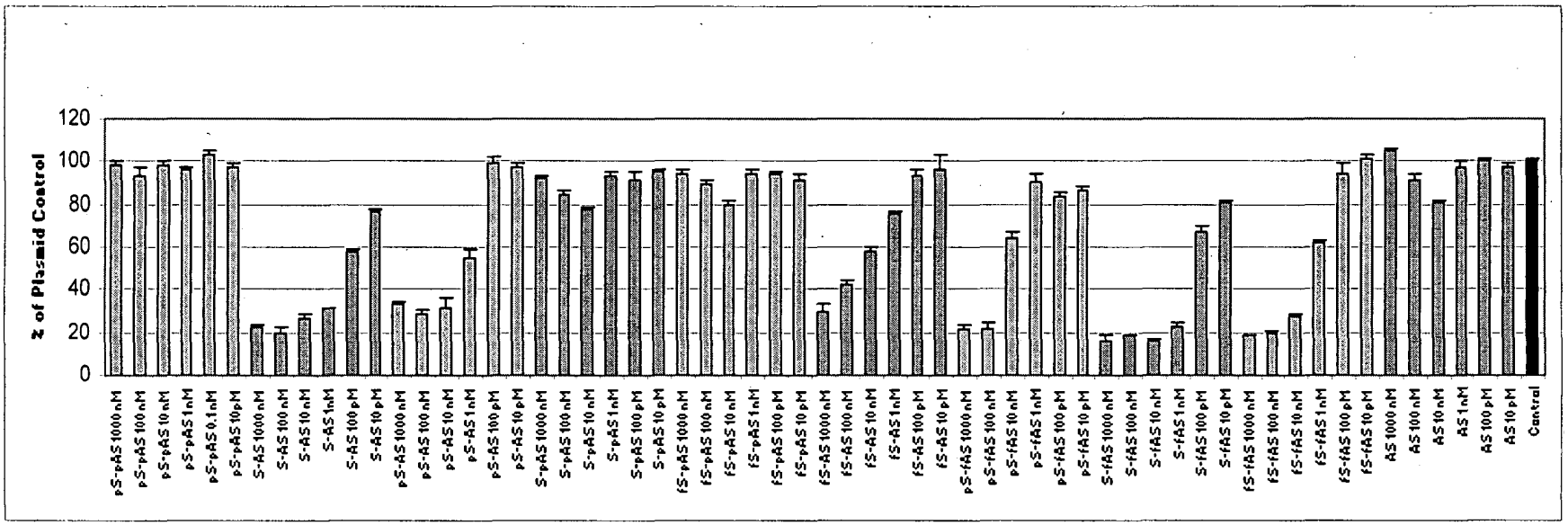


Figure 2A. Time course of orthoester and 2'F modified siRNAs in cell culture, 24 hour time point.

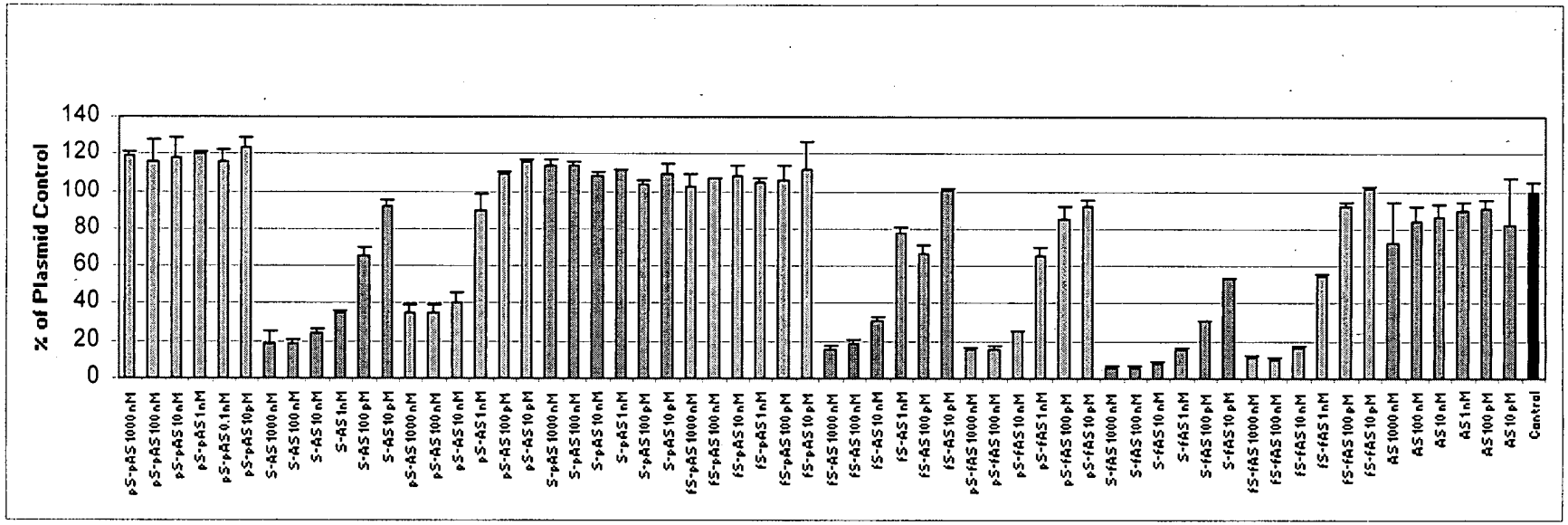


Figure 2B. Time course of orthoester and 2'F modified siRNAs in cell culture, 72 hour time point.

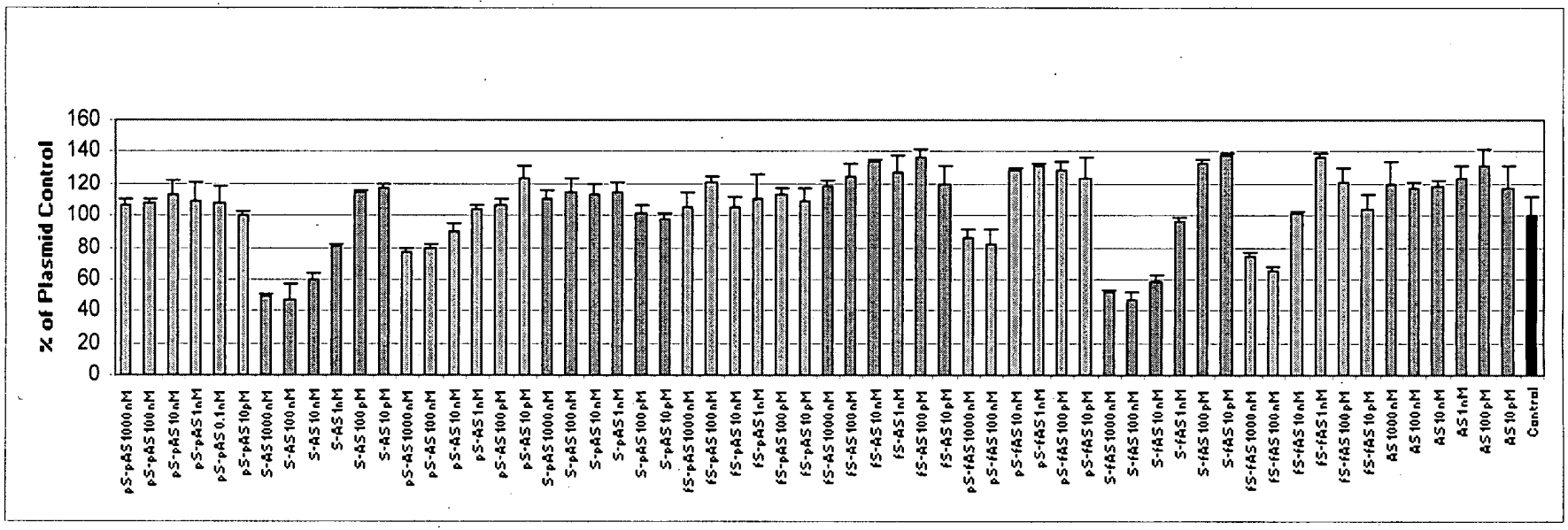


Figure 2C. Time course of orthoester and 2'F modified siRNAs in cell culture, 144 hour time point.

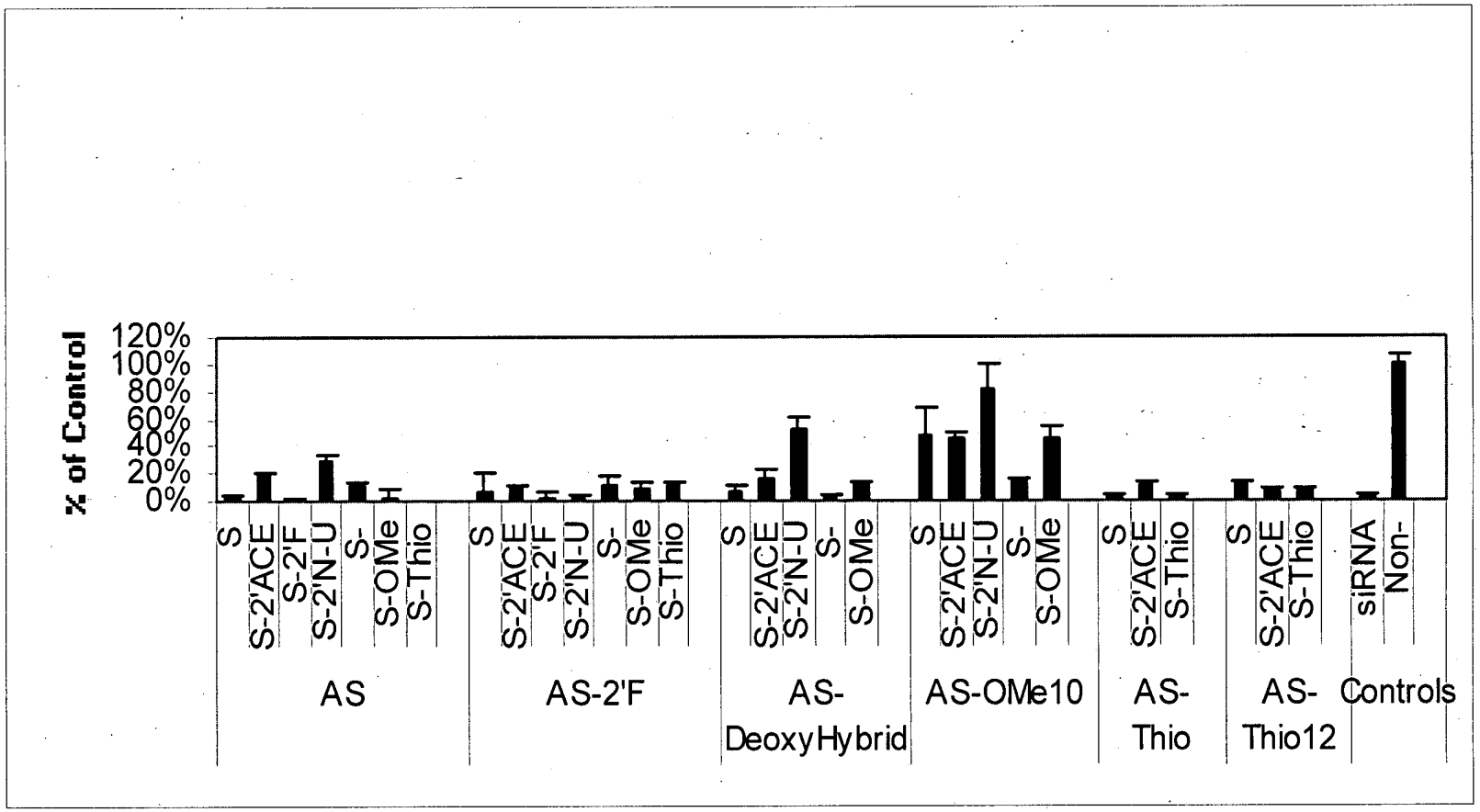


Figure 3. Modifications tolerance in siRNA: sense screen.

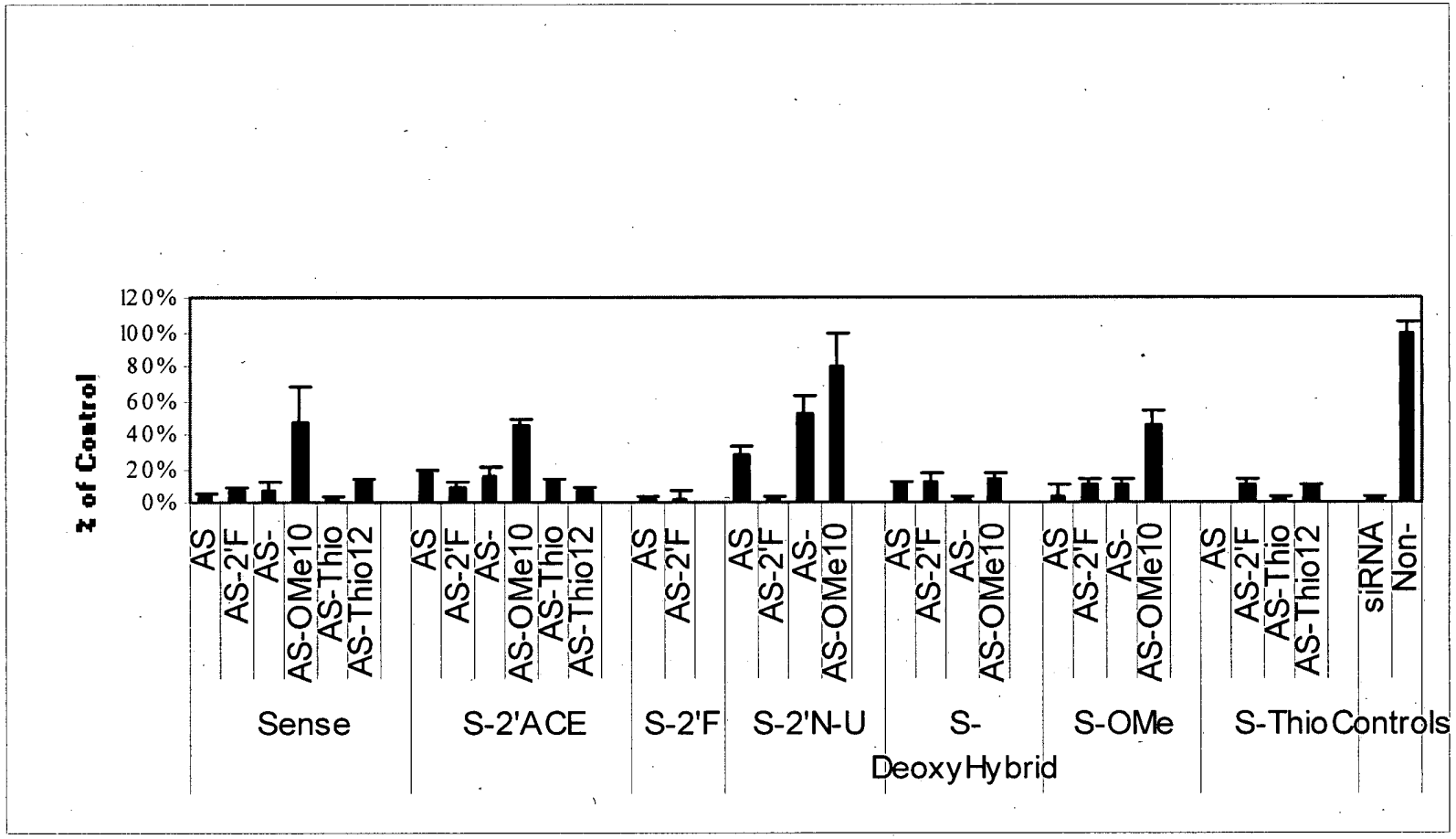


Figure 4. Modifications tolerance in siRNA: antisense screen.

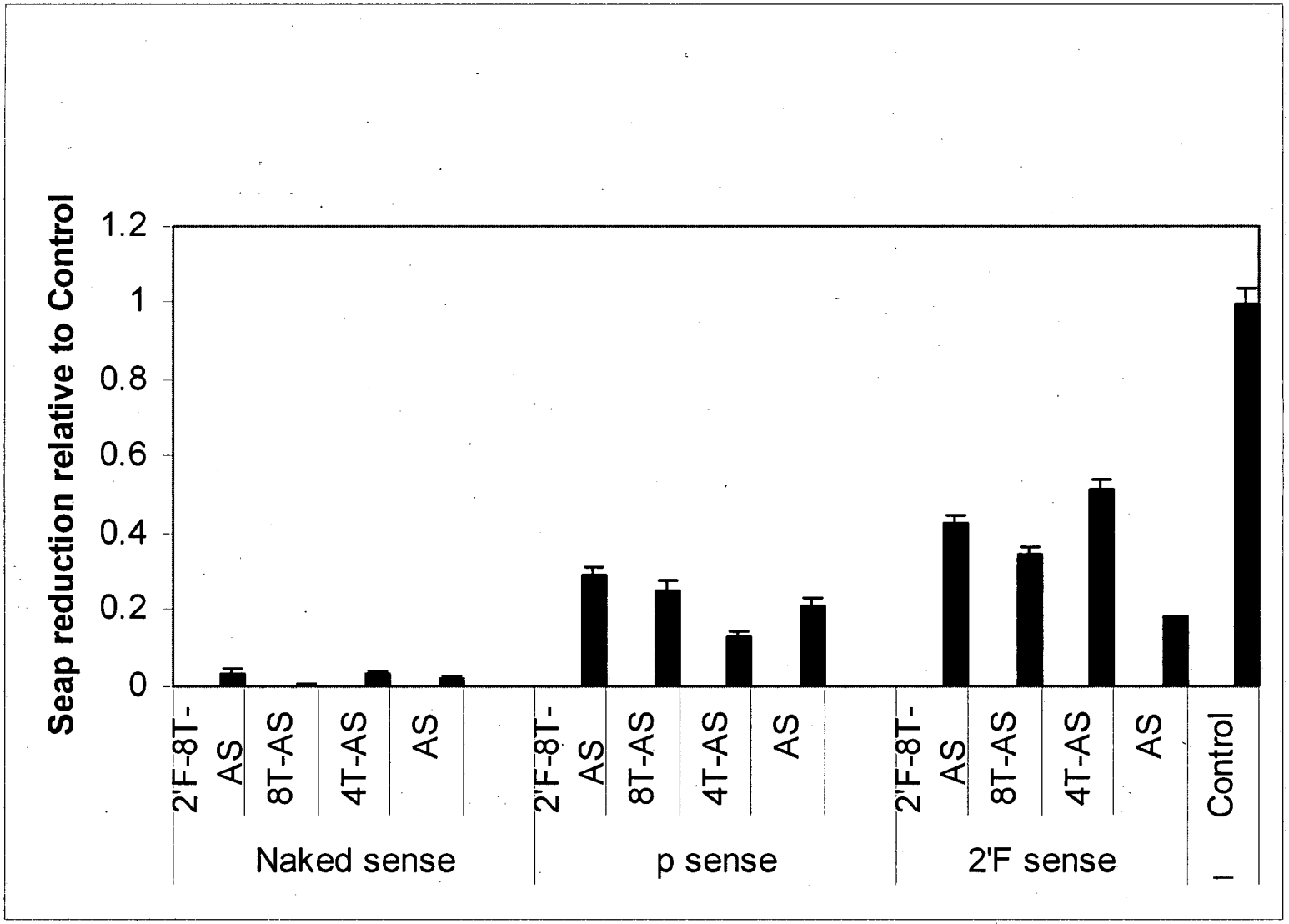


Figure 5. Thio-Based Modifications on the antisense strand

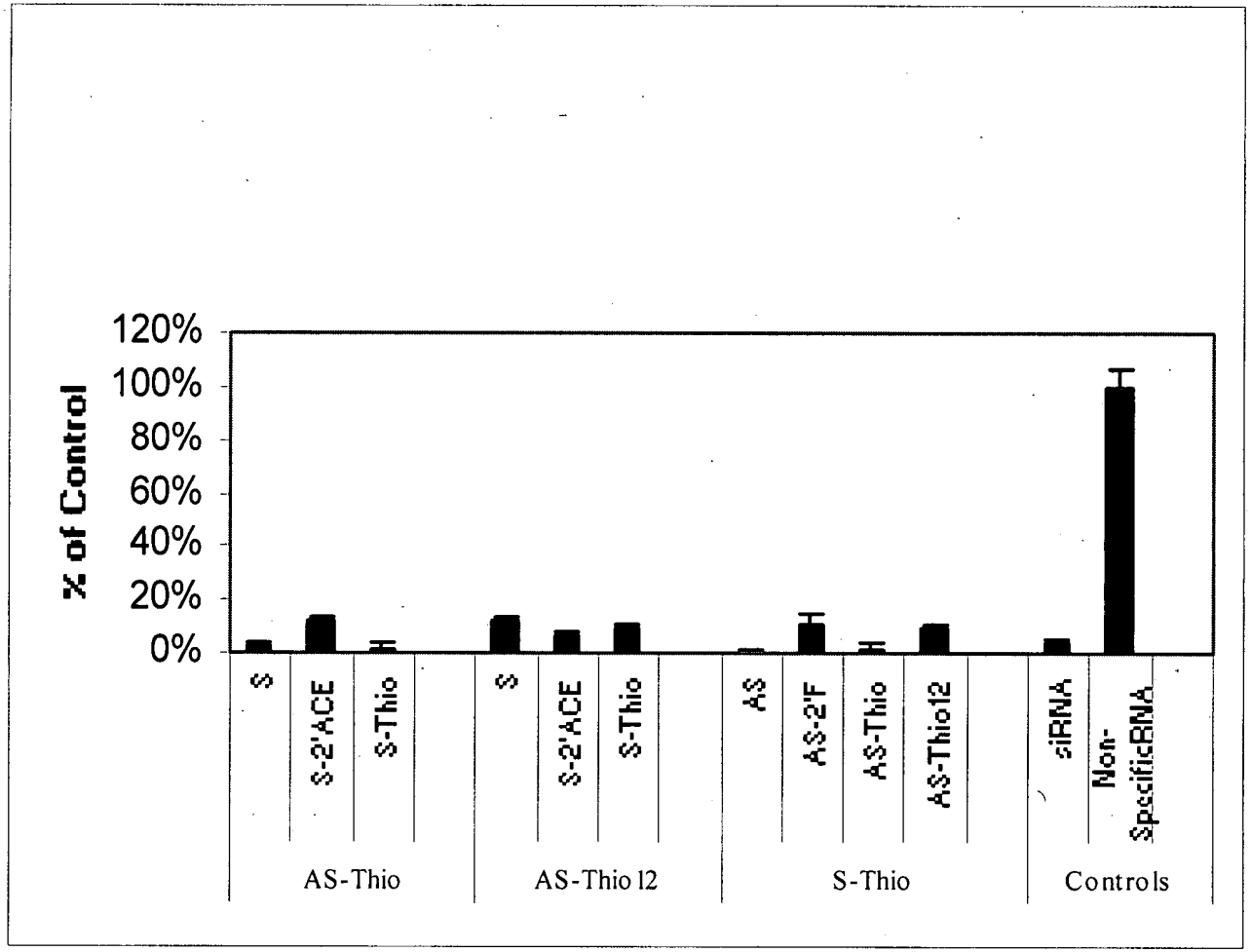


Figure 6. Phosphorothioate modifications are tolerable in both sense and antisense strands

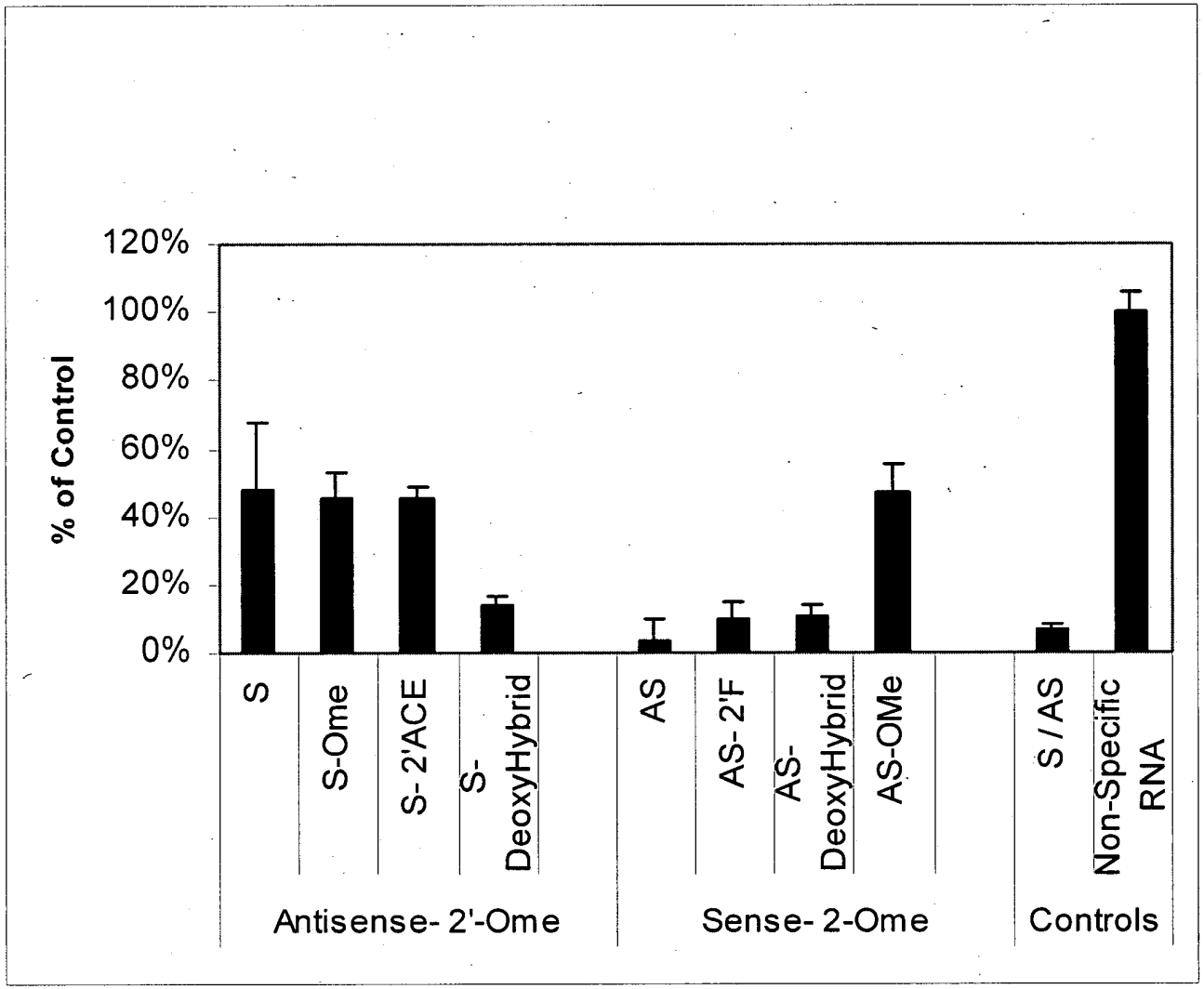


Figure 7. 2'-O-Methyl modifications in RNA interference.

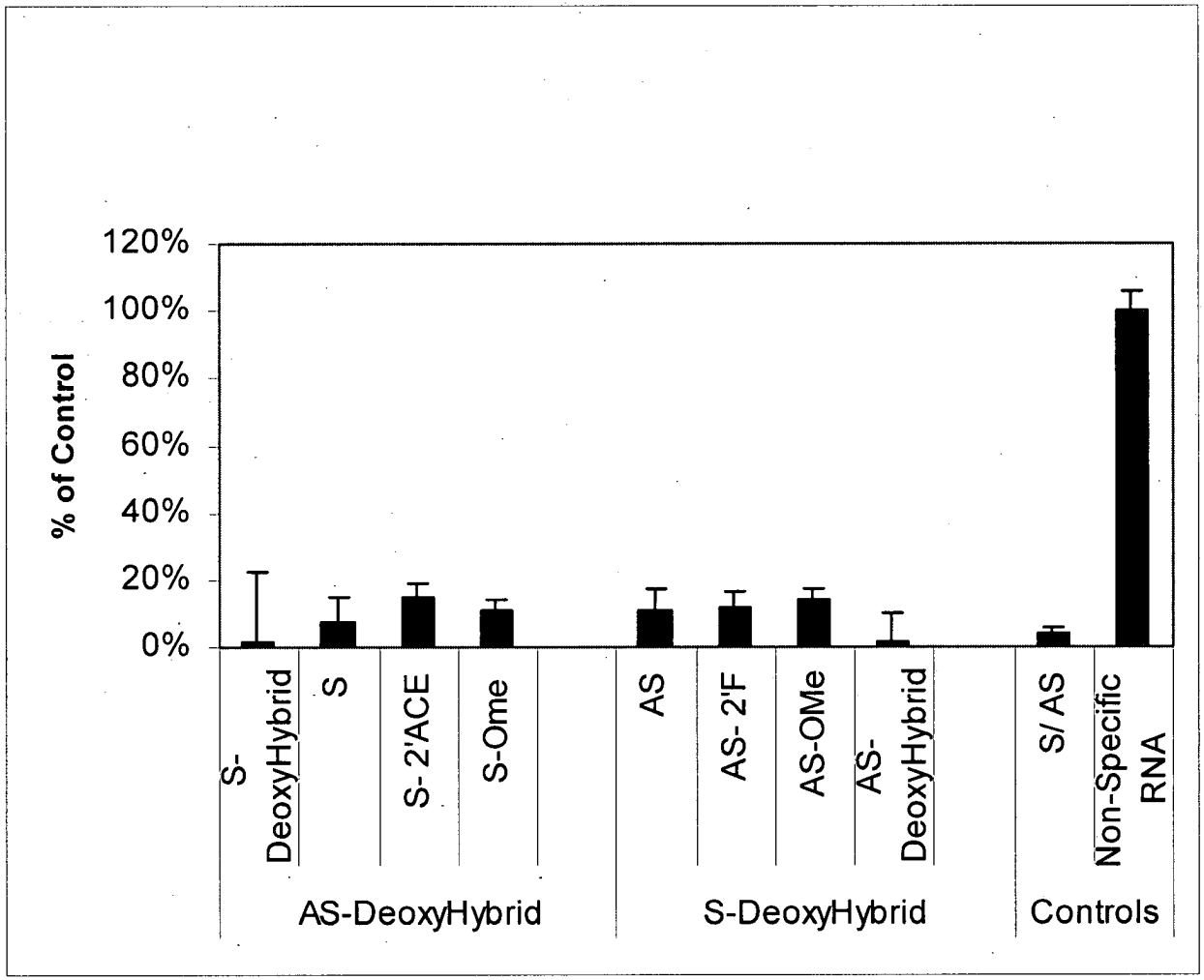


Figure 8. 2'Deoxy-ribo hybrids in RNA intereference

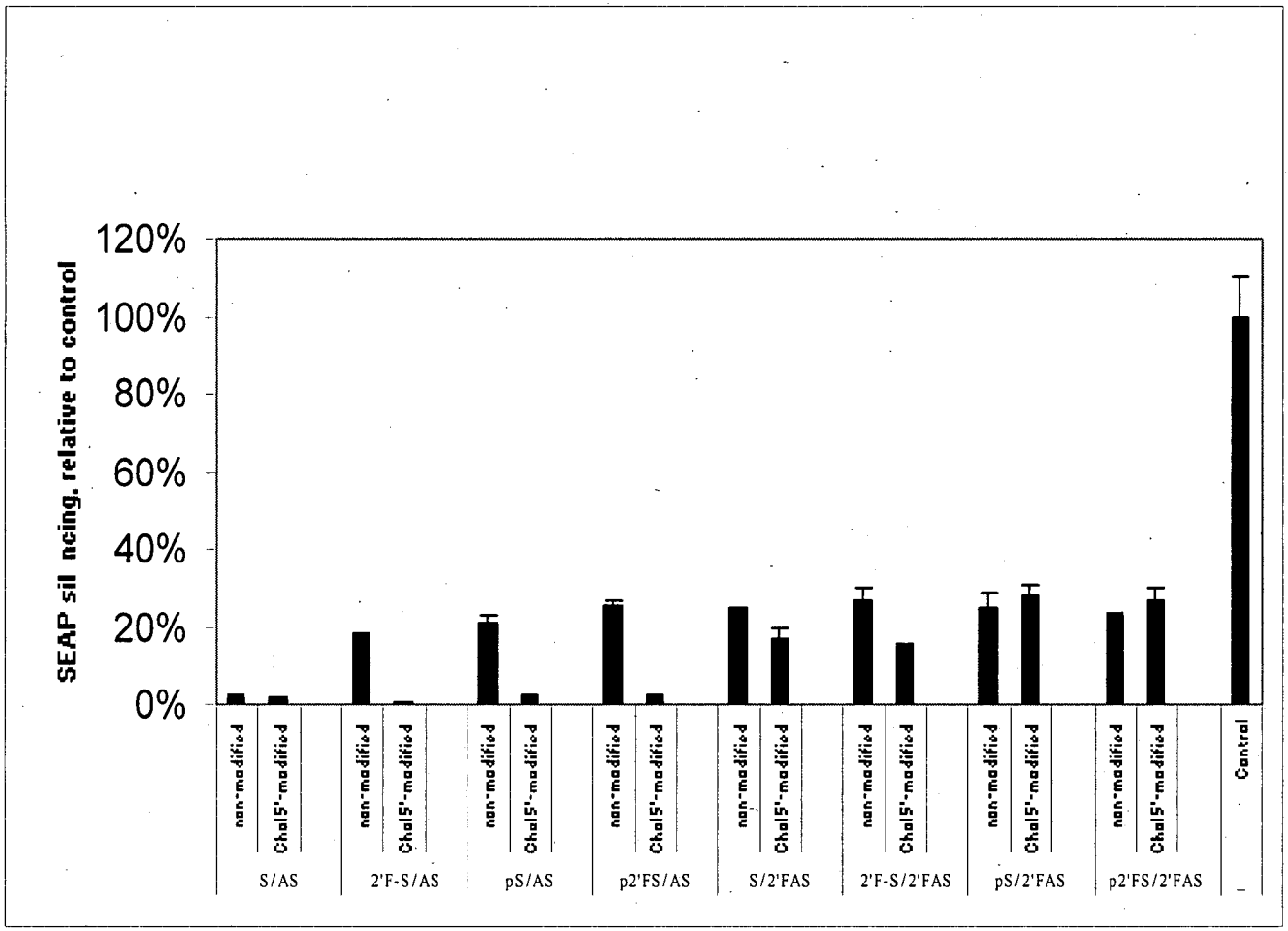


Figure 9. A cholesterol conjugate on the 5' end of the sense strand of an siRNA duplex increases potency of modified siRNA

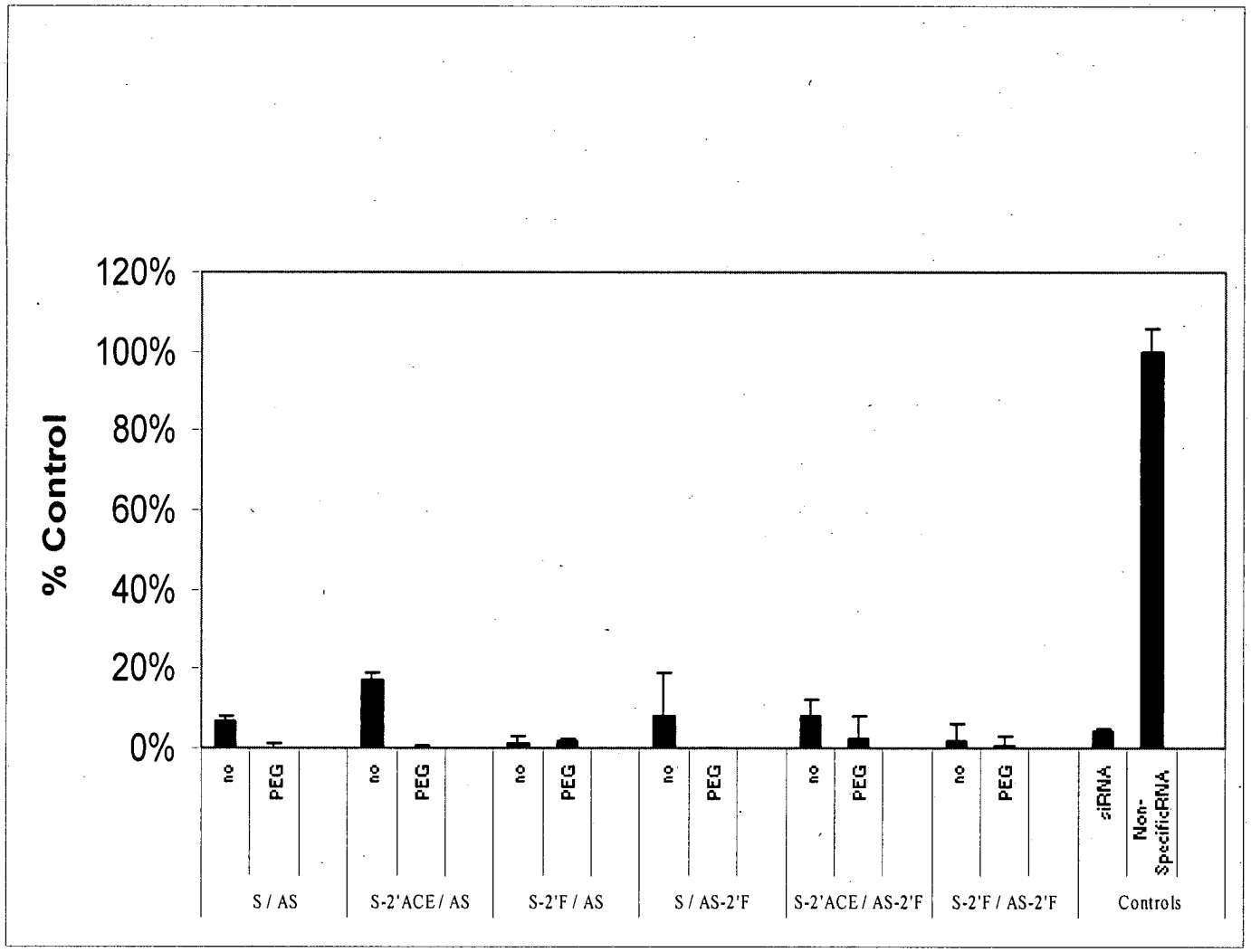


Figure 10. A PEG conjugate on the 5' end of the sense strand of an siRNA duplex increases siRNA potency

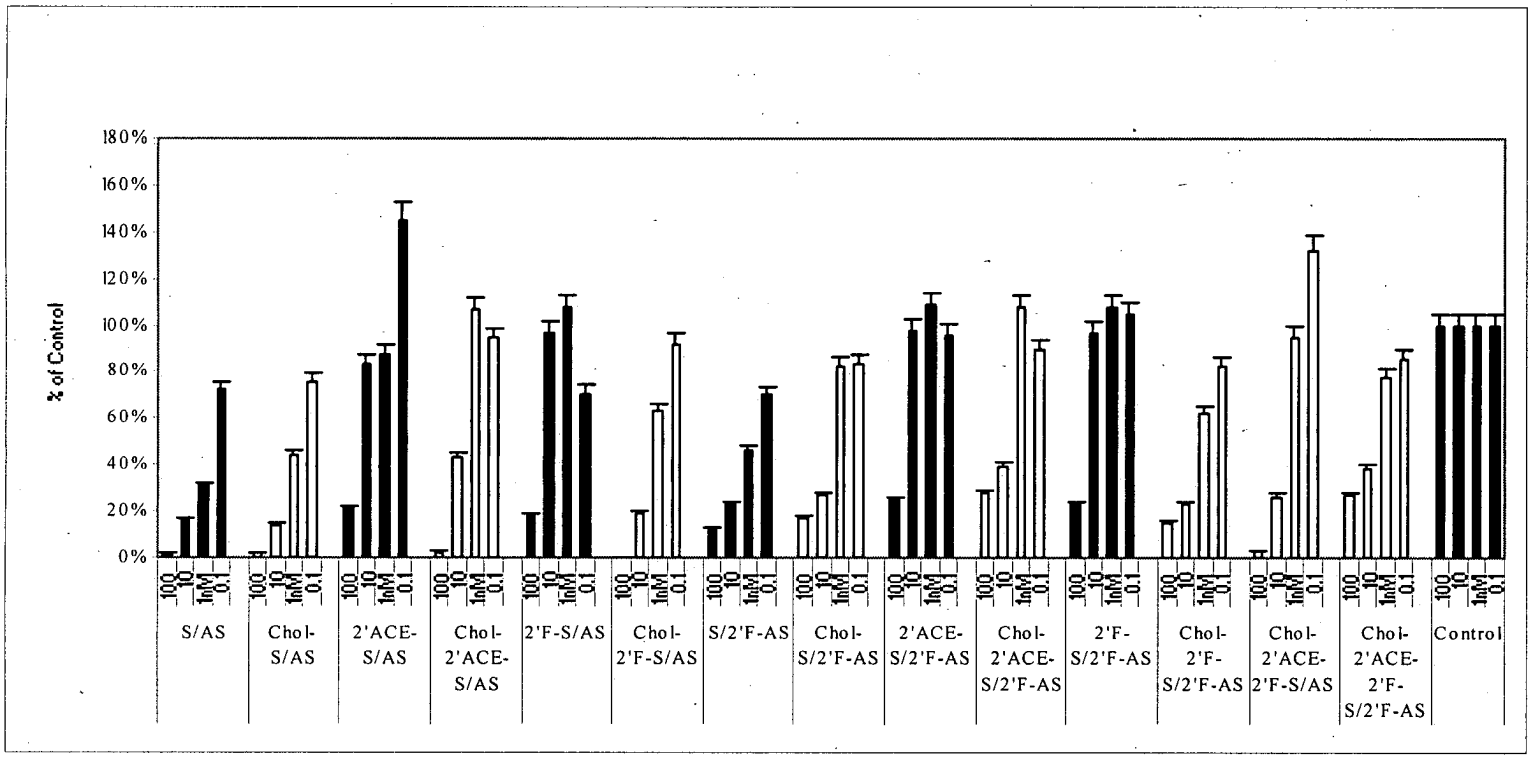


Figure 11. A sense strand having a 5' cholesterol conjugate results in increased potency and decreased dose of 2'F and orthoester modified oligos

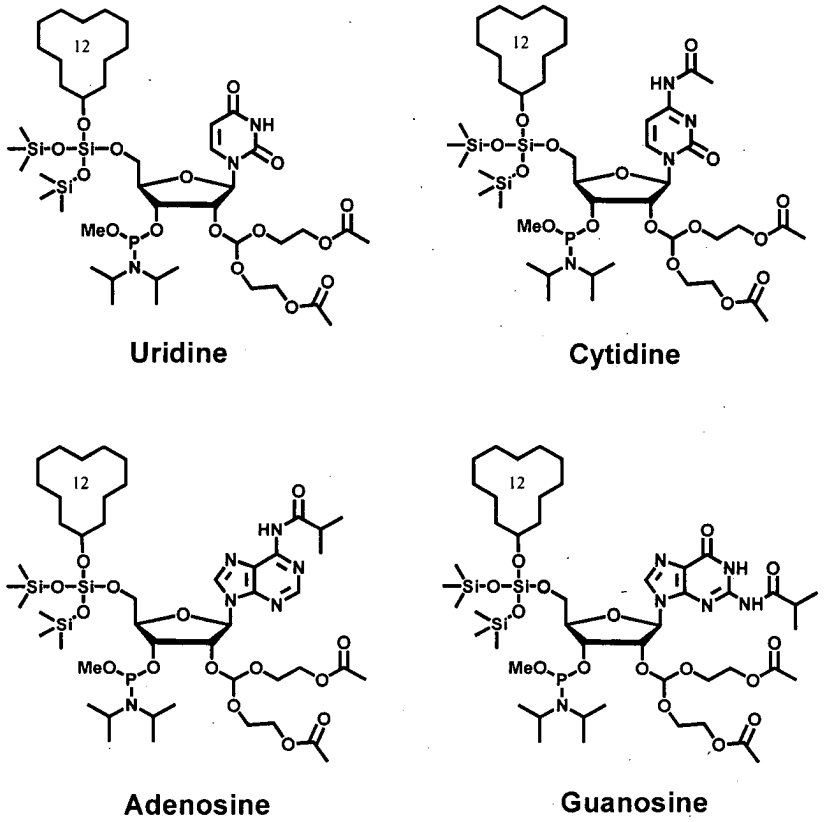
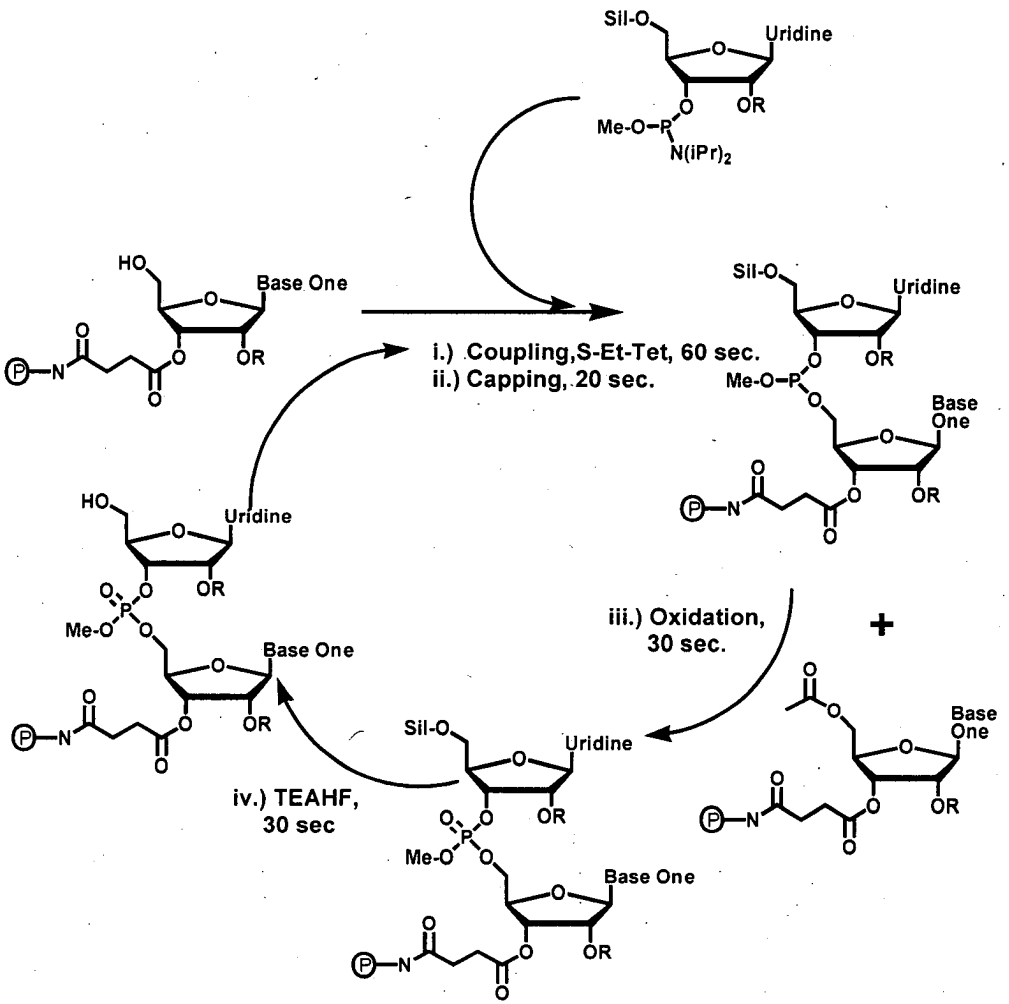


Figure 12: Protected RNA nucleoside phosphoramidites for Dharmacon 2'-ACE RNA synthesis chemistry.



(i) Couple next nucleoside with S-ethyl-tetrazole catalyst, 60 seconds
(ii) Cap unreacted 5′-hydroxyls, 20 seconds
(iii) Oxidize phosphorus linkage (t-butyl hydroperoxide)
(iv) 5′-deprotection with triethylammonium fluoride ions (TEAHF), 30 seconds

Figure 13: Outline of Dharmacon RNA Synthesis Cycle.

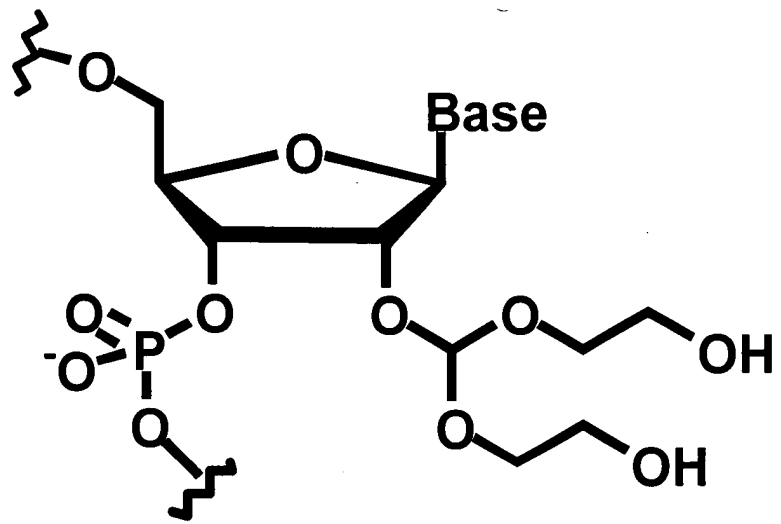


Figure 14: Structure of 2'-ACE protected RNA immediately prior to 2'-deprotection.

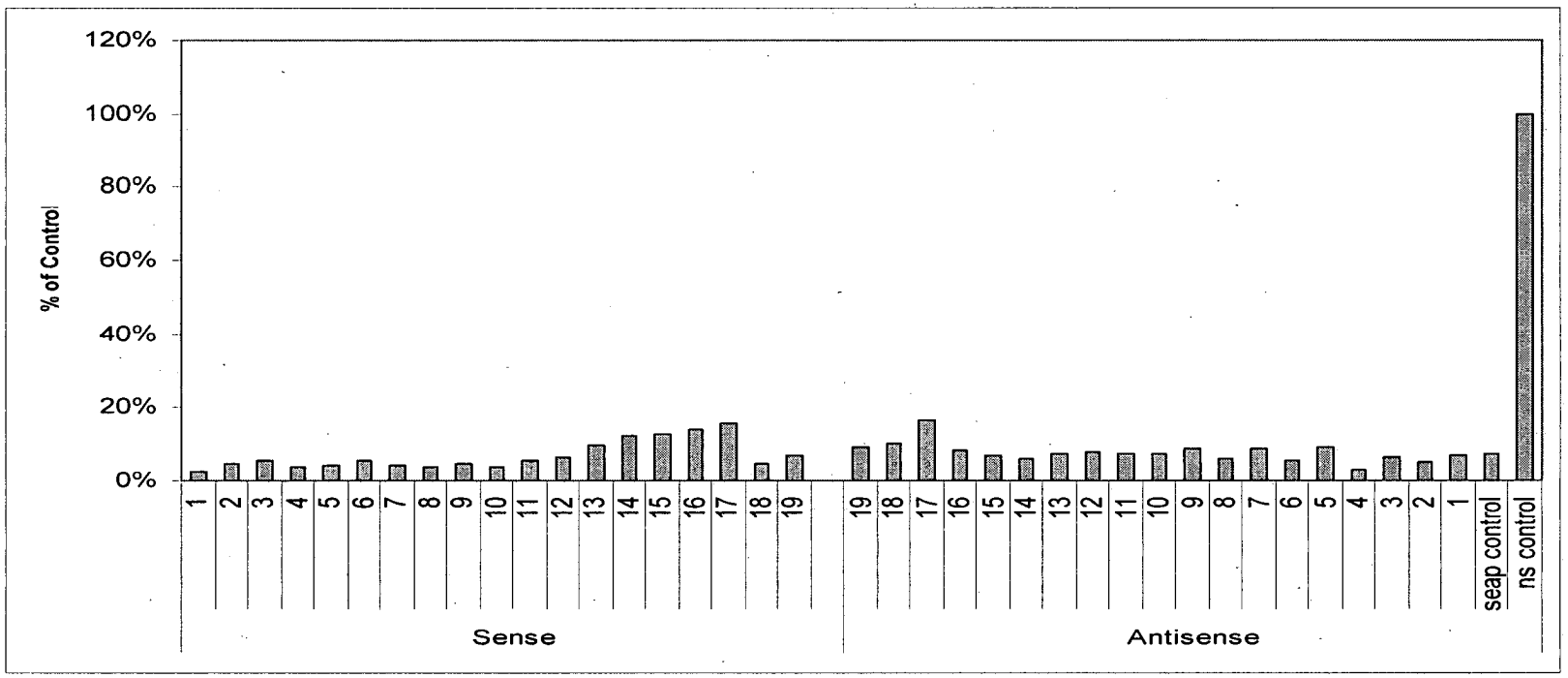


Figure 15A: Single Deoxynucleotide Modification

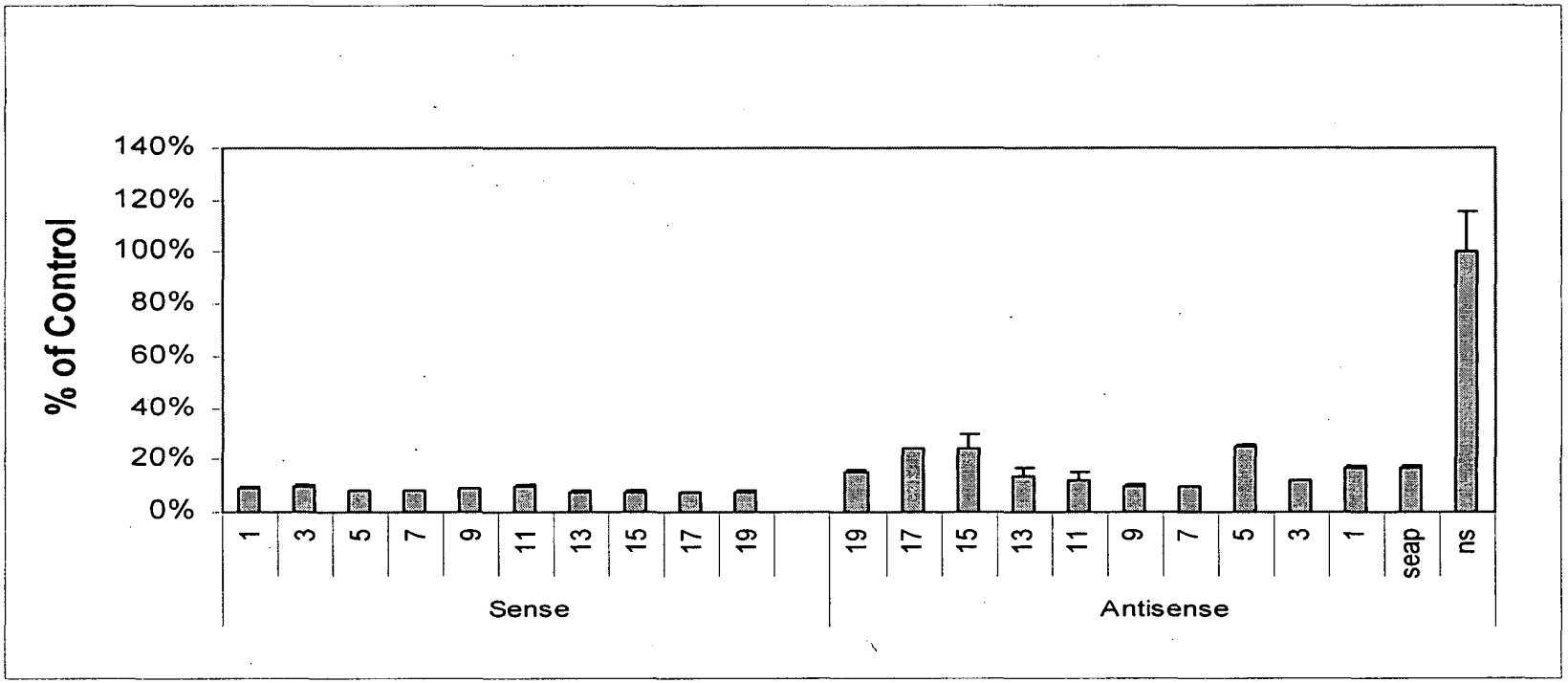


Figure 15B: Two Deoxynucleotide Modifications in Tandem

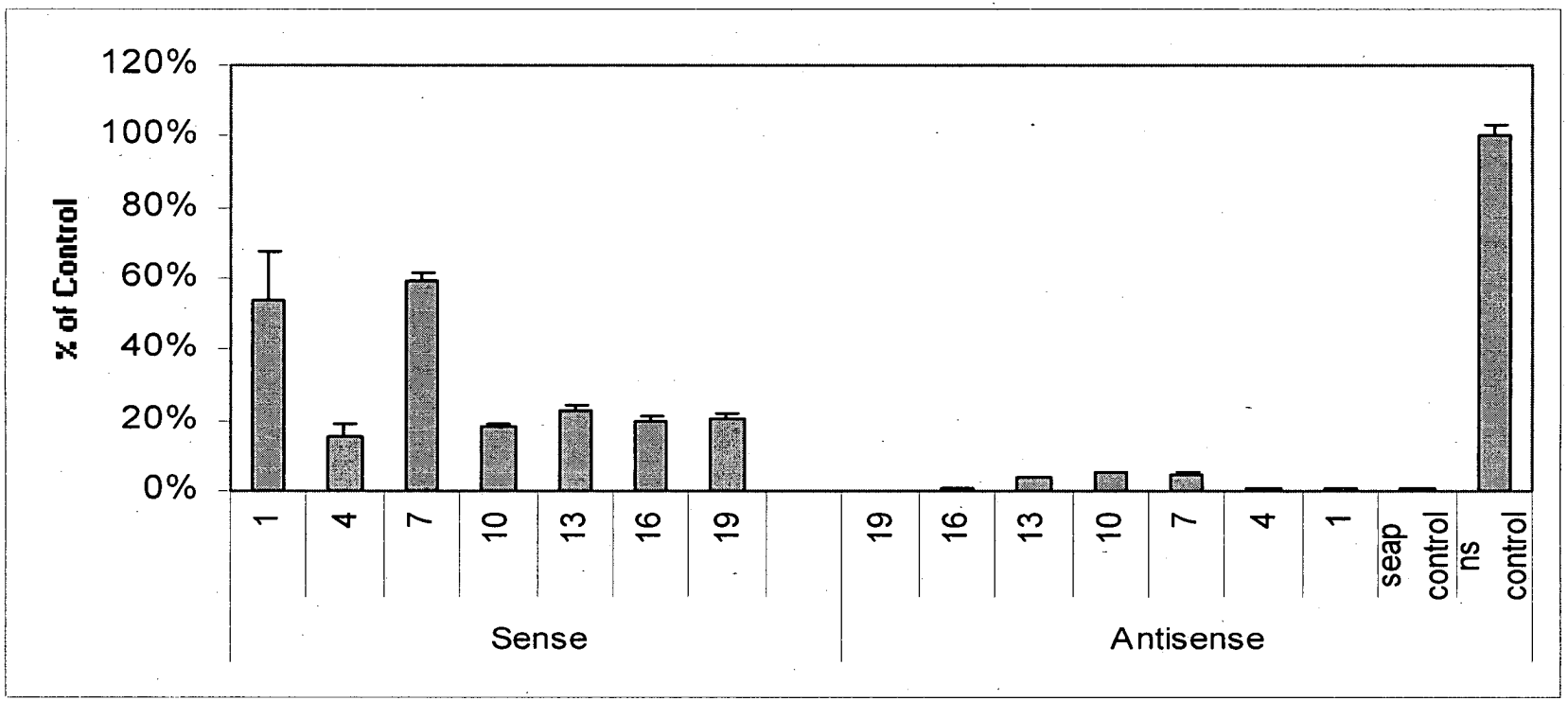


Figure 15C: Three Deoxynucleotide Modifications in Tandem

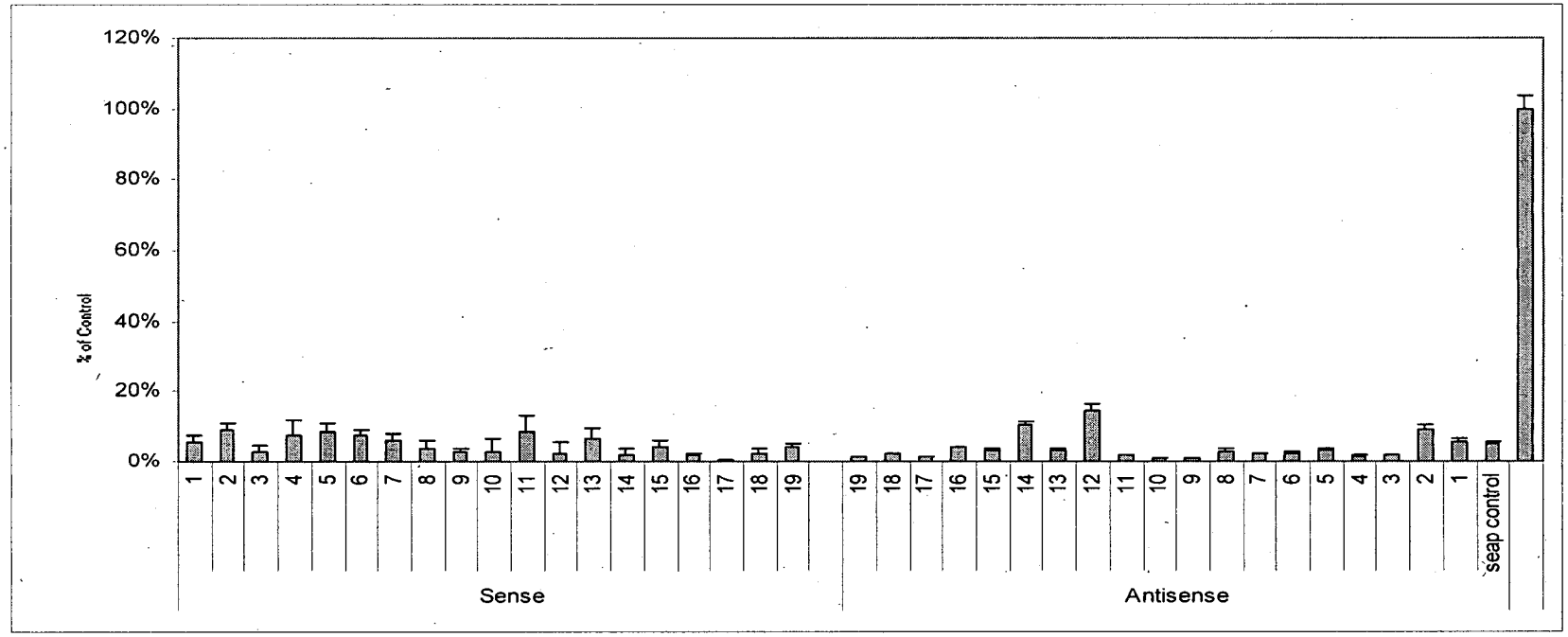


Figure 16A: Single 2'-O-Methyl Modification

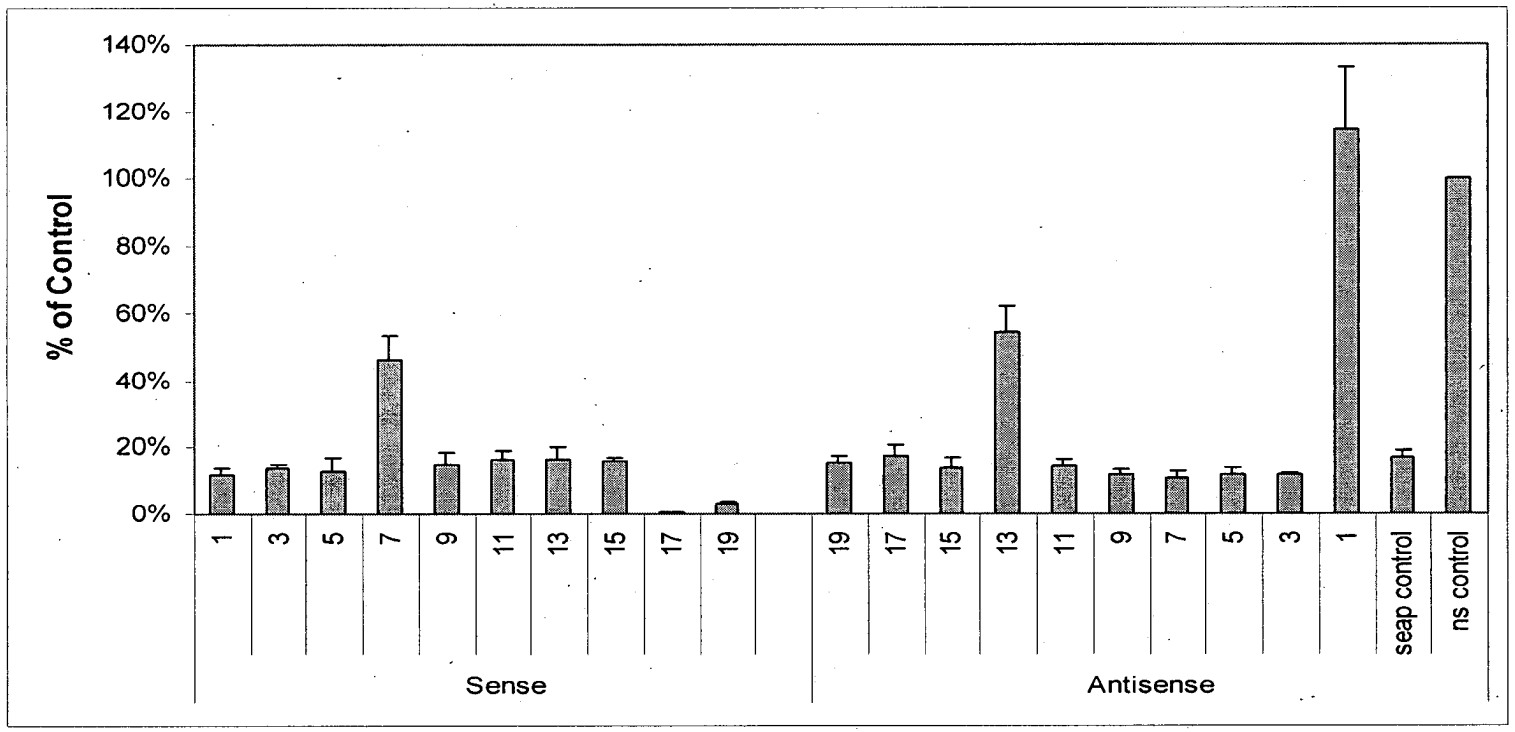


Figure 16B: Two 2'-O-Methyl Modifications in Tandem

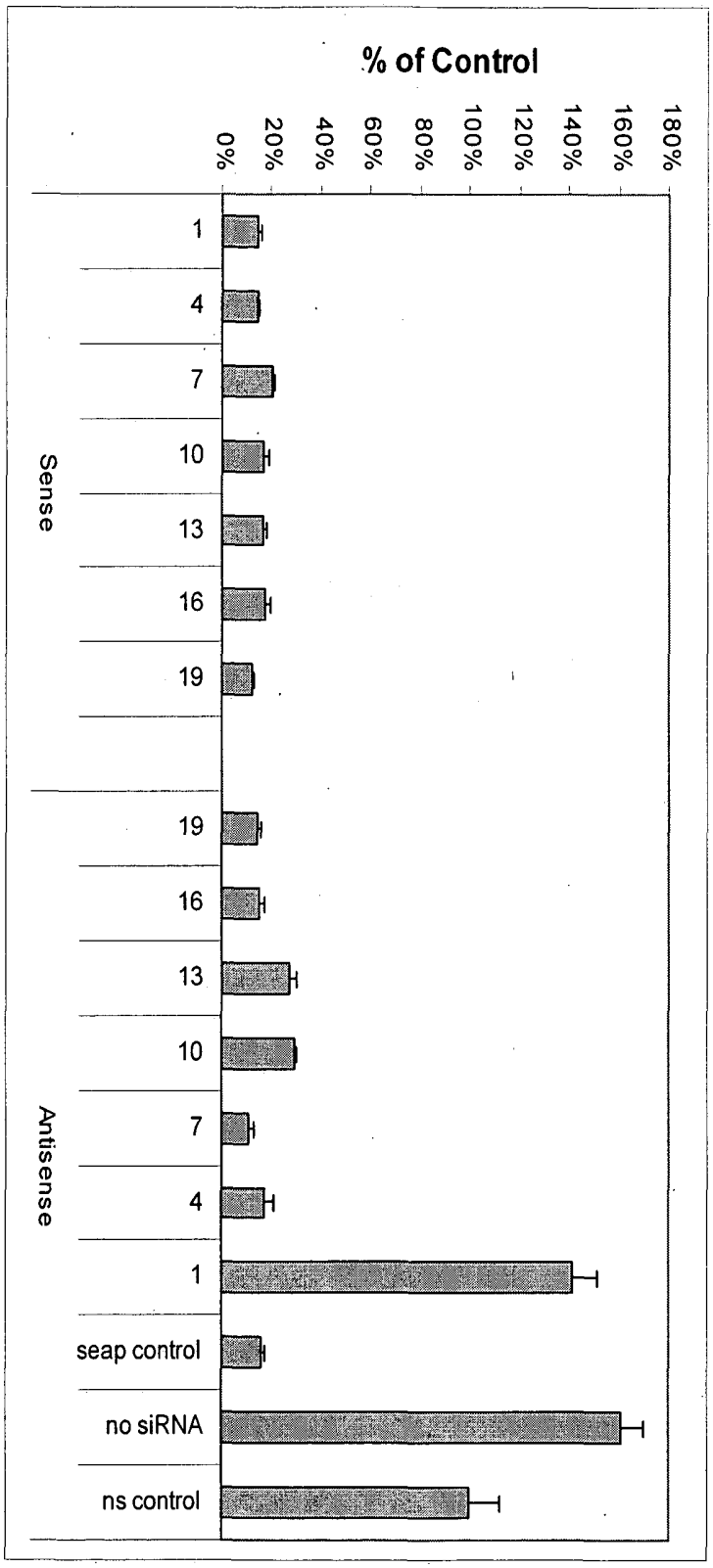


Figure 16C: Three 2'-O-Methyl Modifications in Tandem

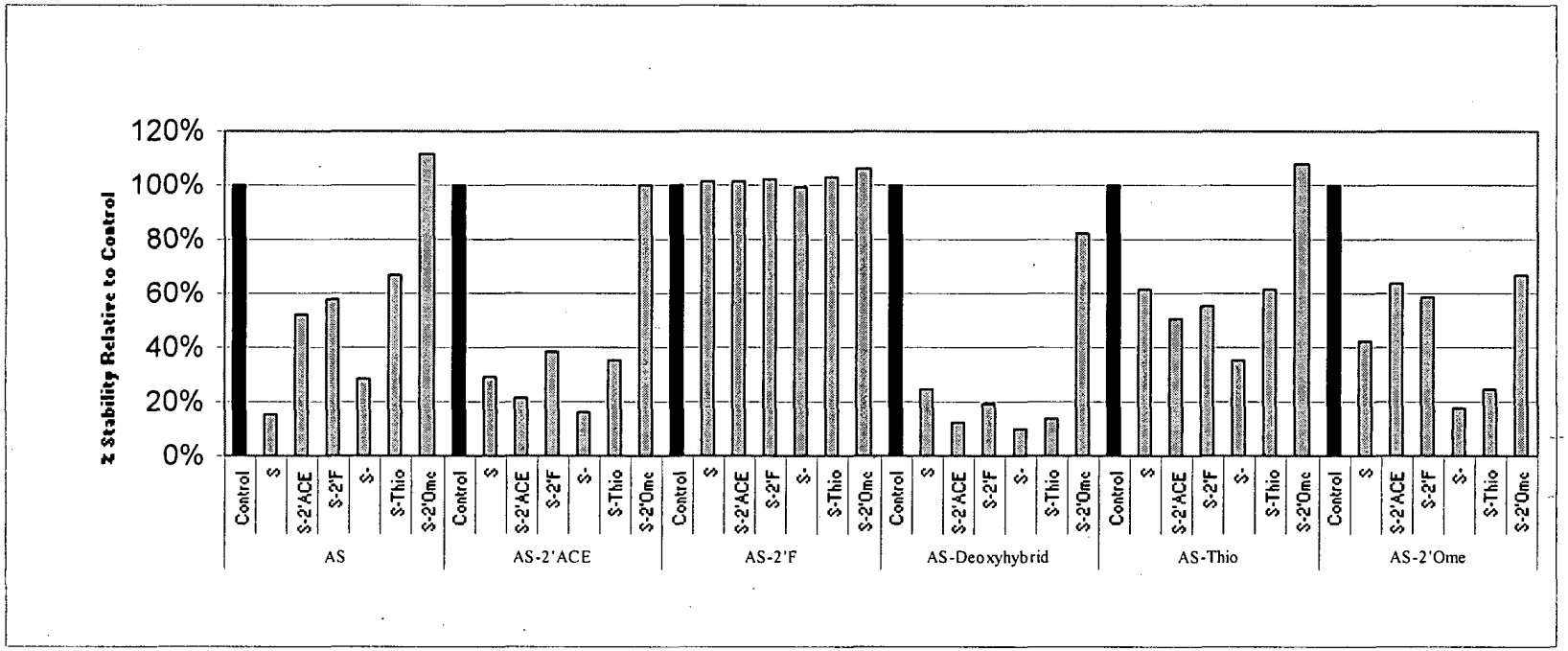


Figure 17: Stability Screen for One Hour Incubation in Media

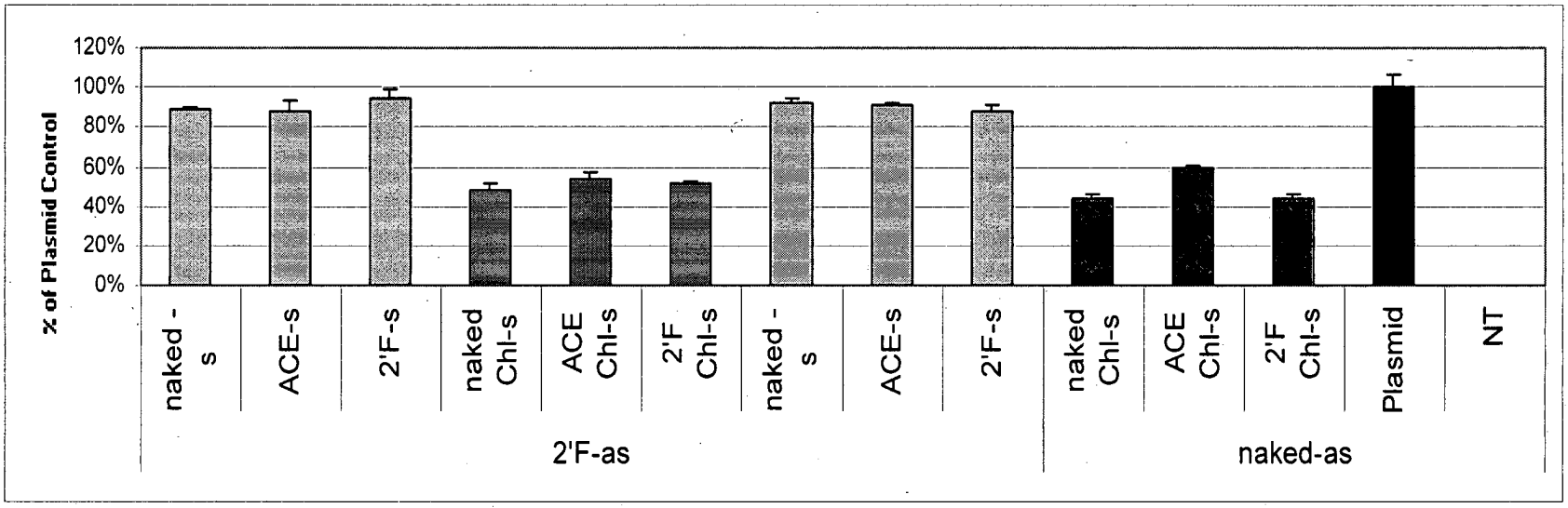


Figure 18: Effect of Cholesterol Modification on Passive Delivery of siRNA

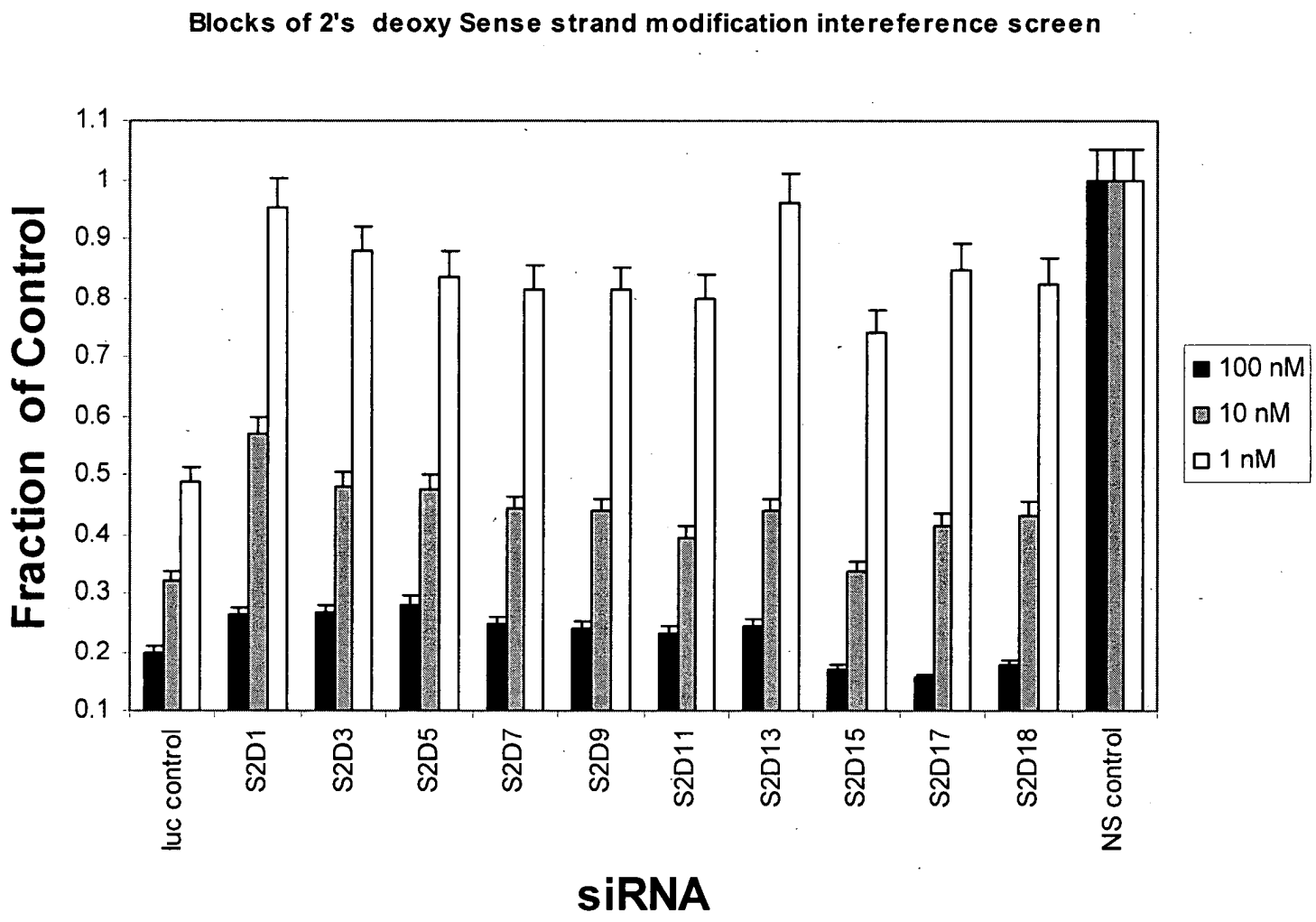


Figure 19: Modification intereference screen: blocks of 2 deoxy in the sense strand

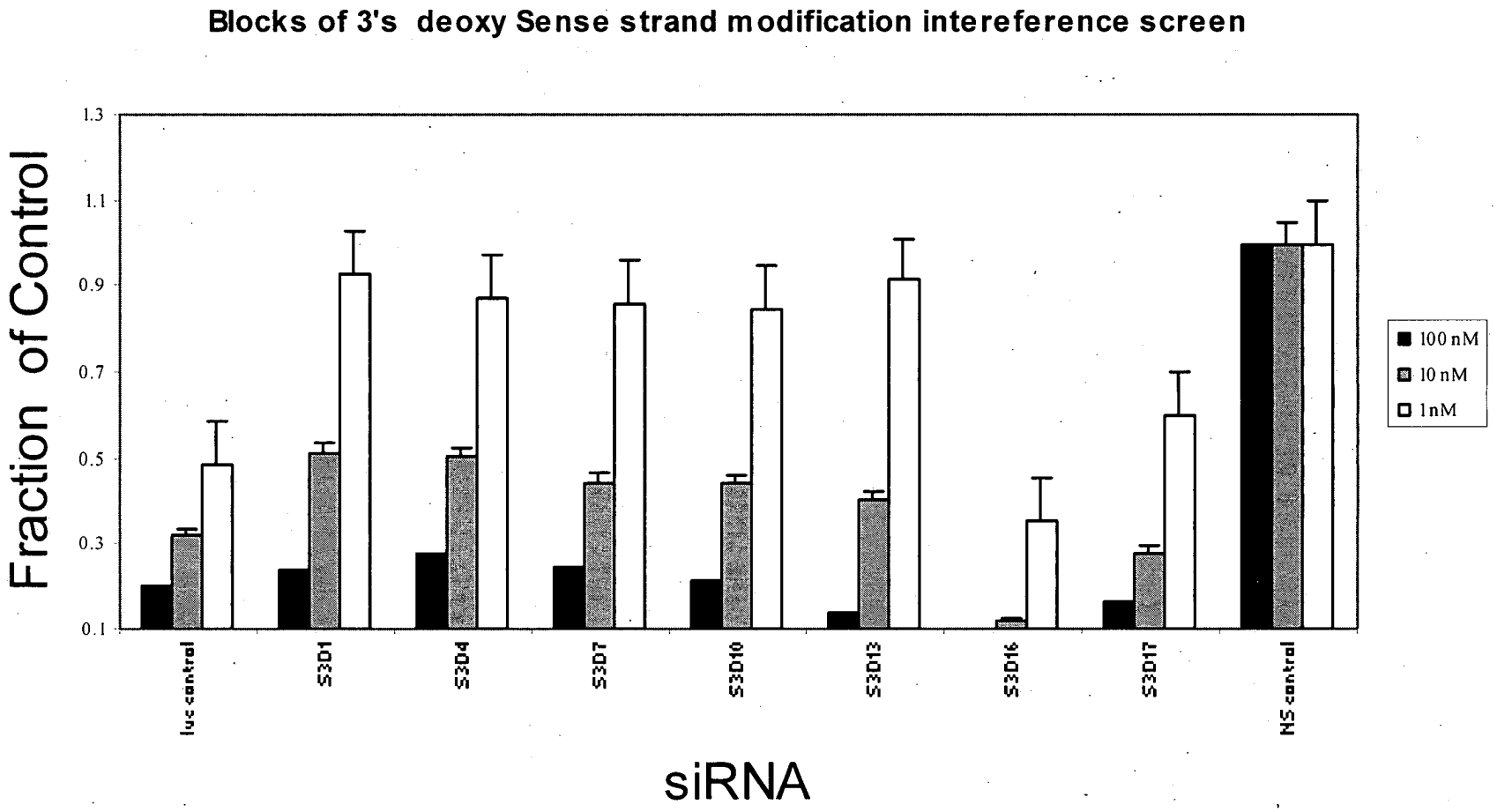


Figure 20: Modification intereference screen: blocks of 3 deoxy in the sense strand

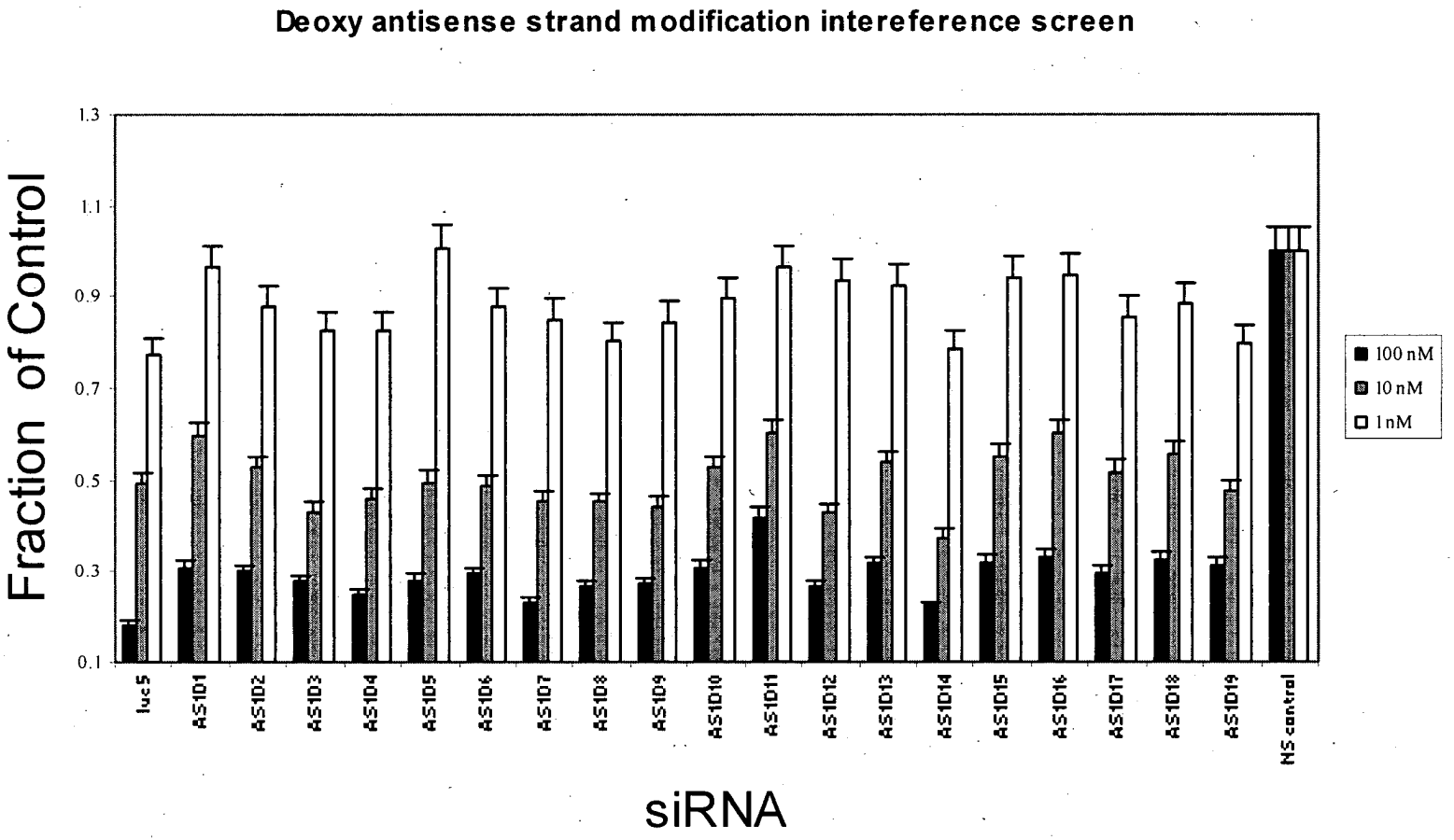


Figure 21: Modification intereference screen: deoxy in the antisense strand

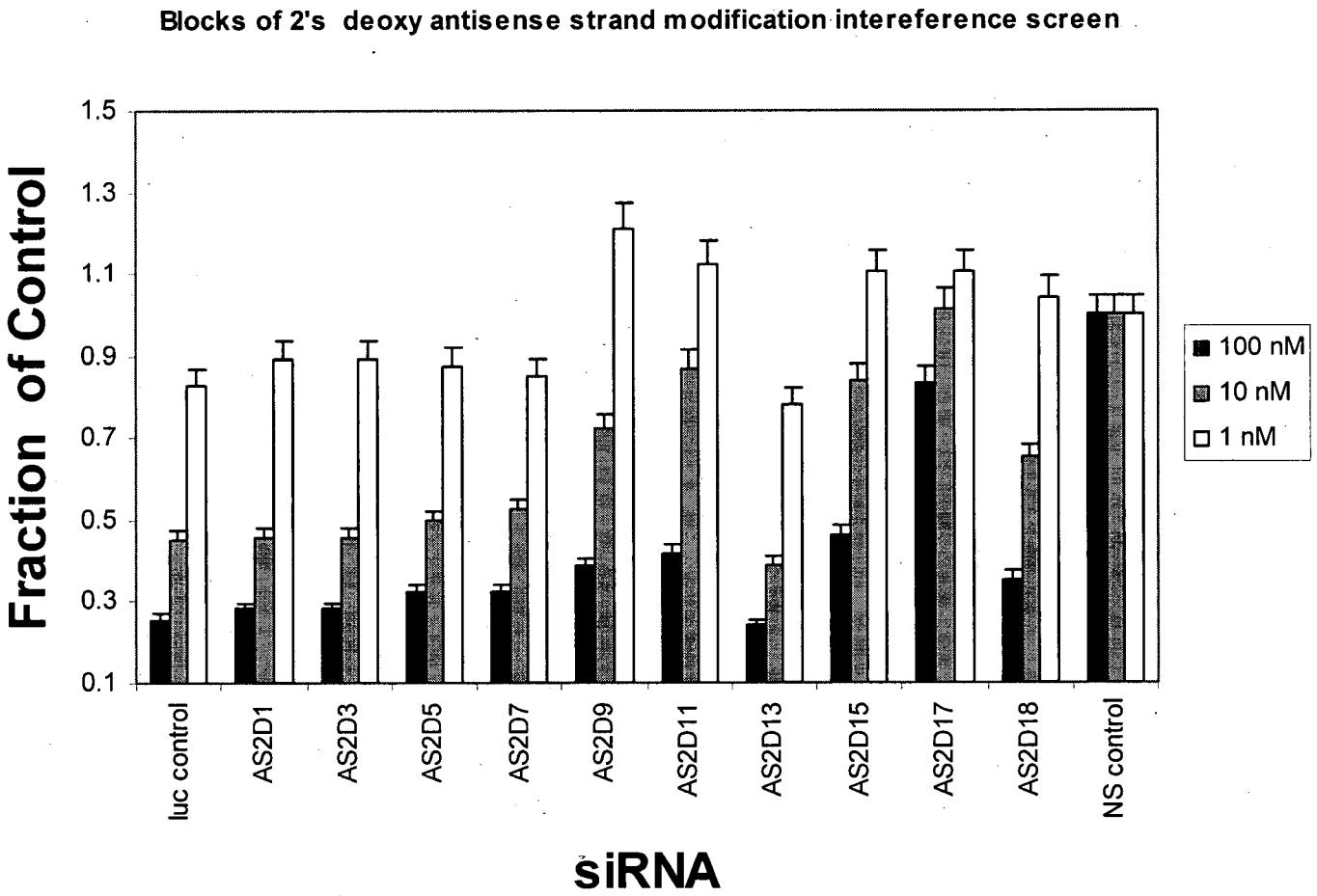


Figure 22: Modification intereference screen: blocks of 2 deoxy in the antisense strand

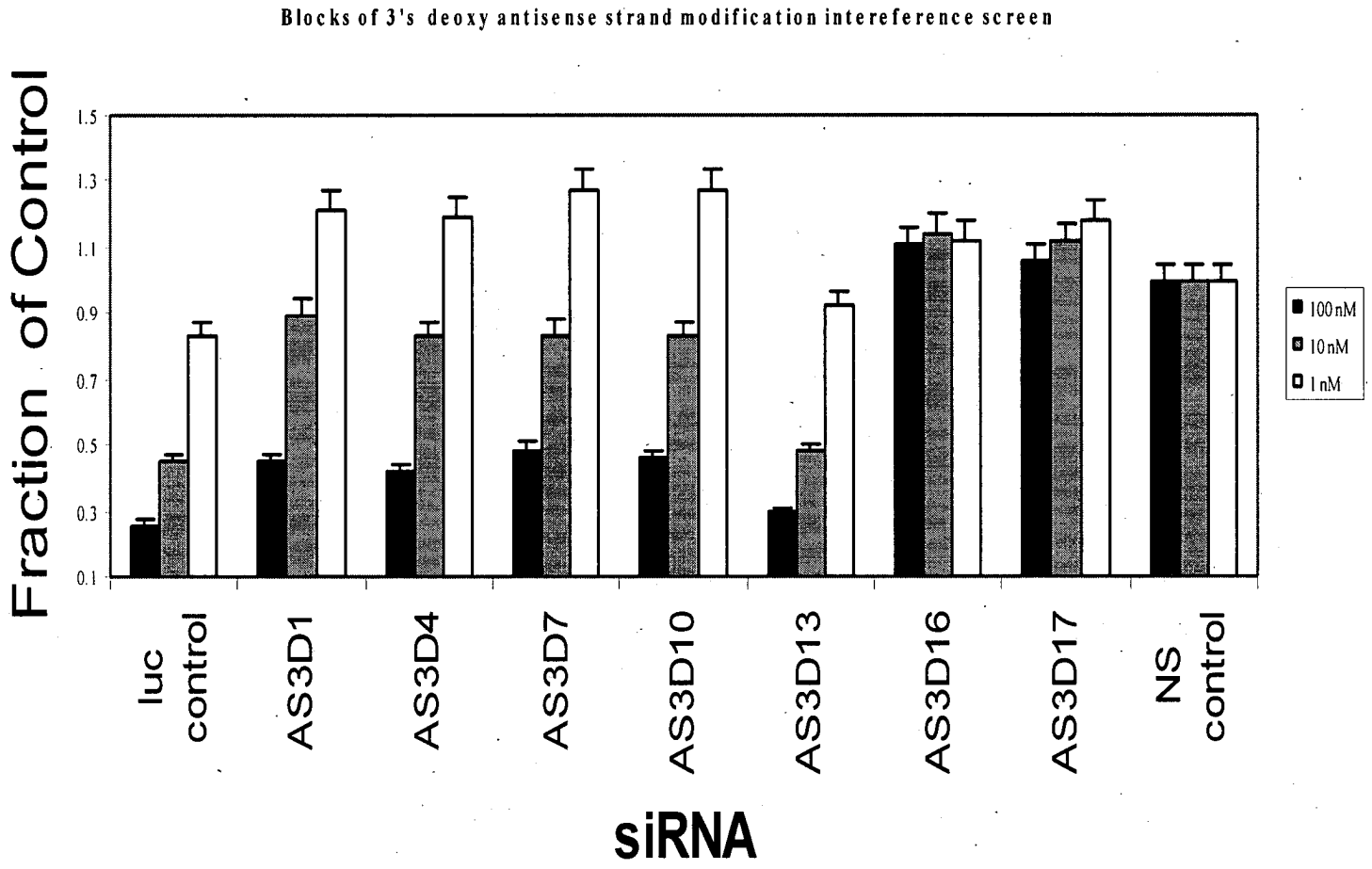


Figure 23: Modification intereference screen: blocks of 3 deoxy in the antisense strand

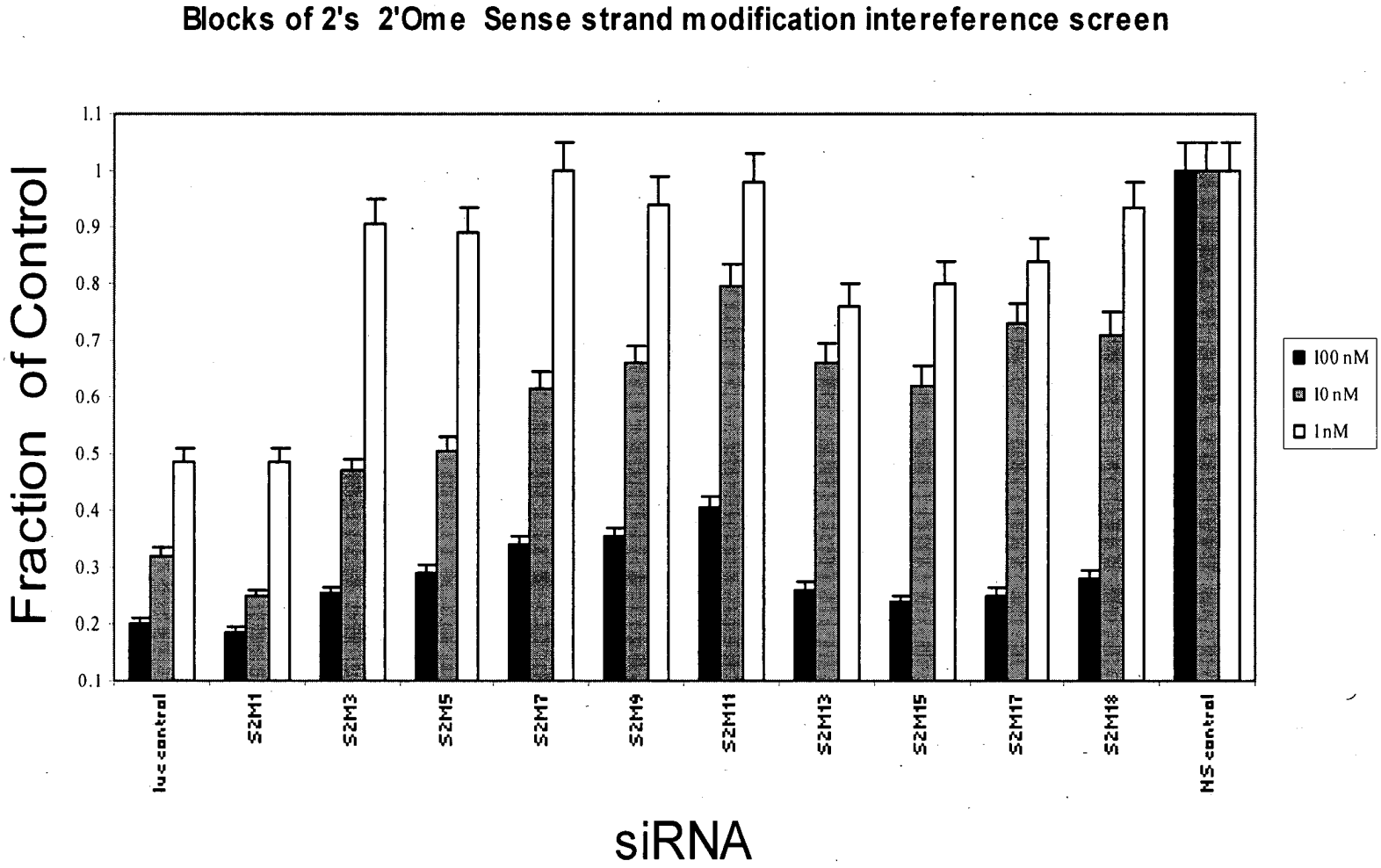


Figure 24: Modification intereference screen: blocks of 2 methoxy in the sense strand

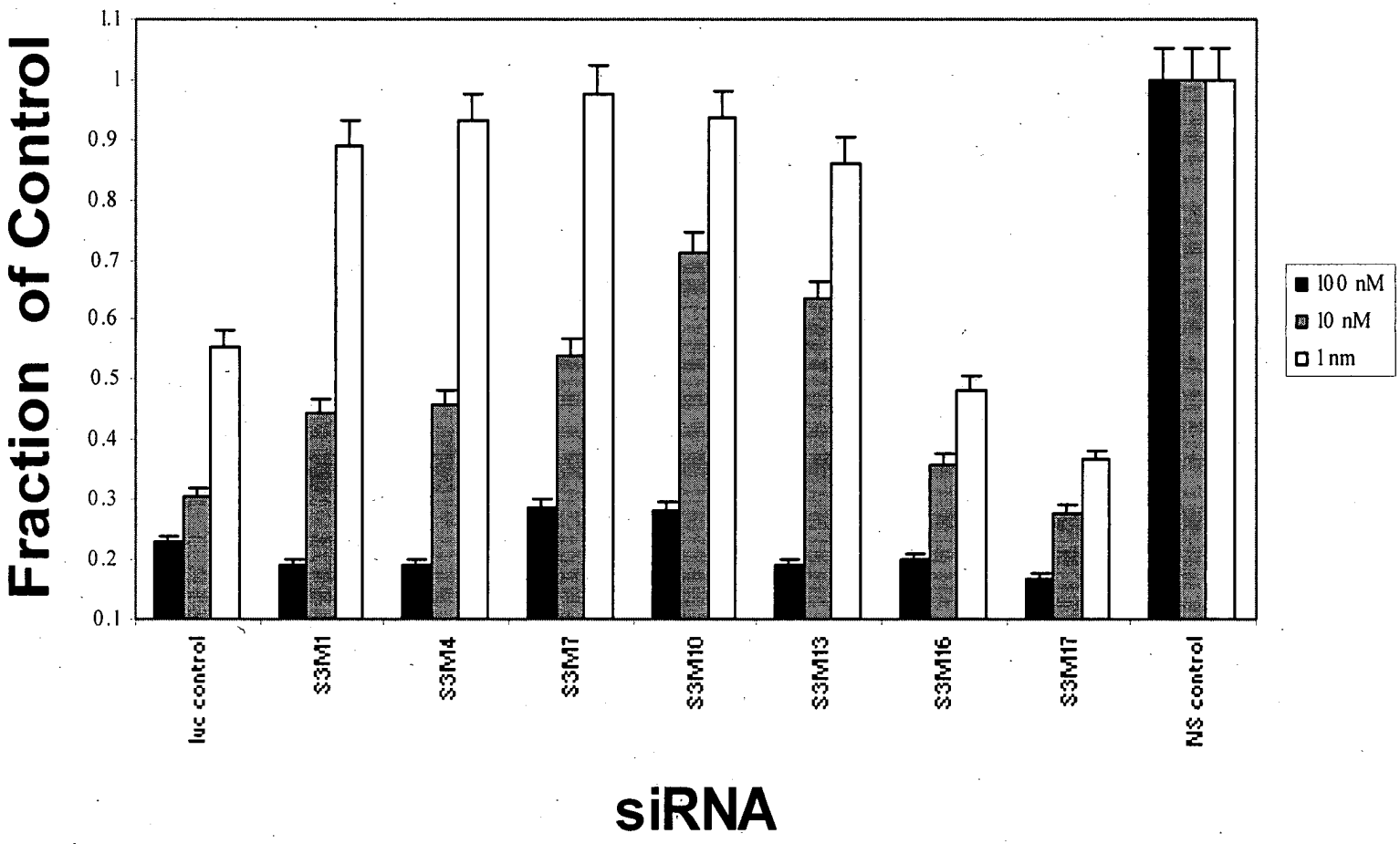


Figure 25: Modification intereference screen: blocks of 3 methoxy in the sense strand

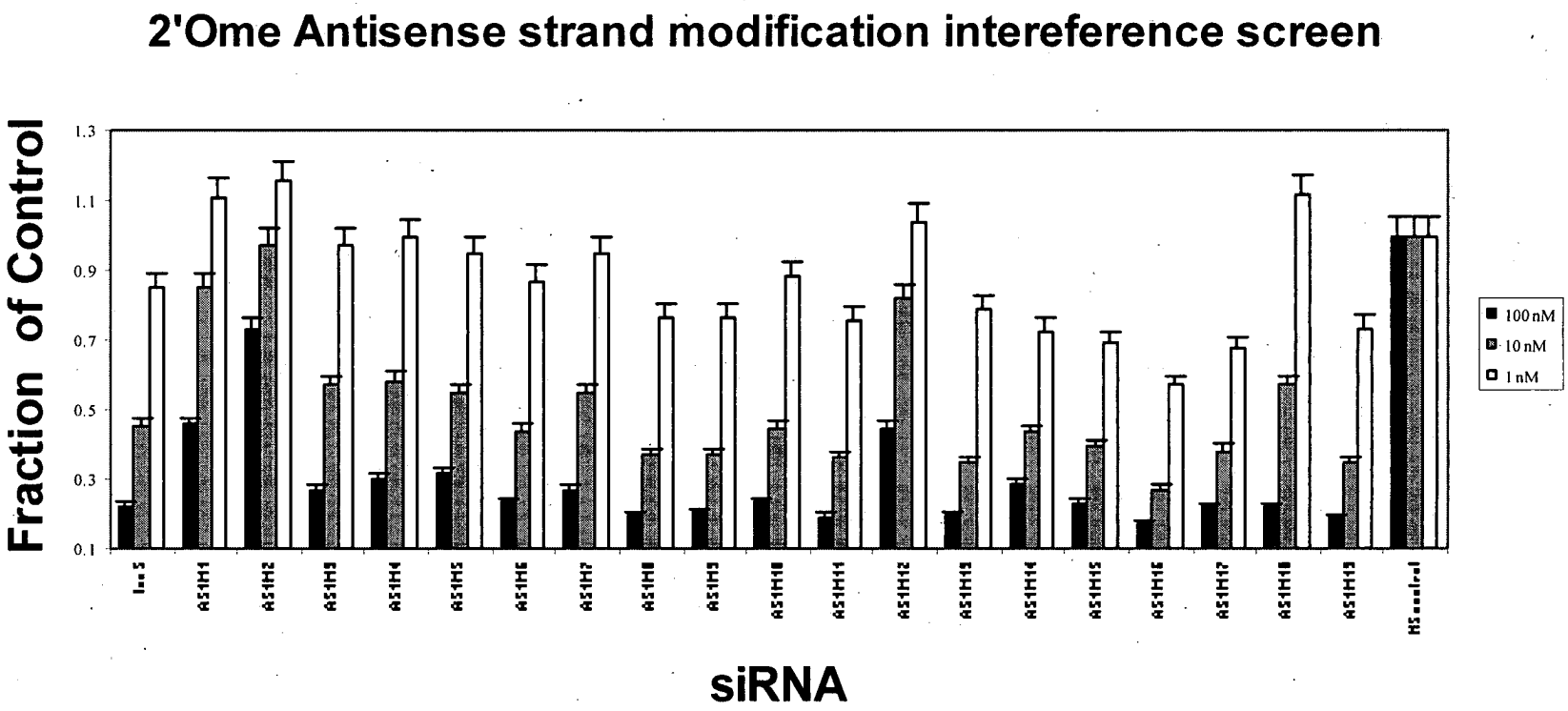


Figure 26: Modification intereference screen: methoxy in the antisense strand

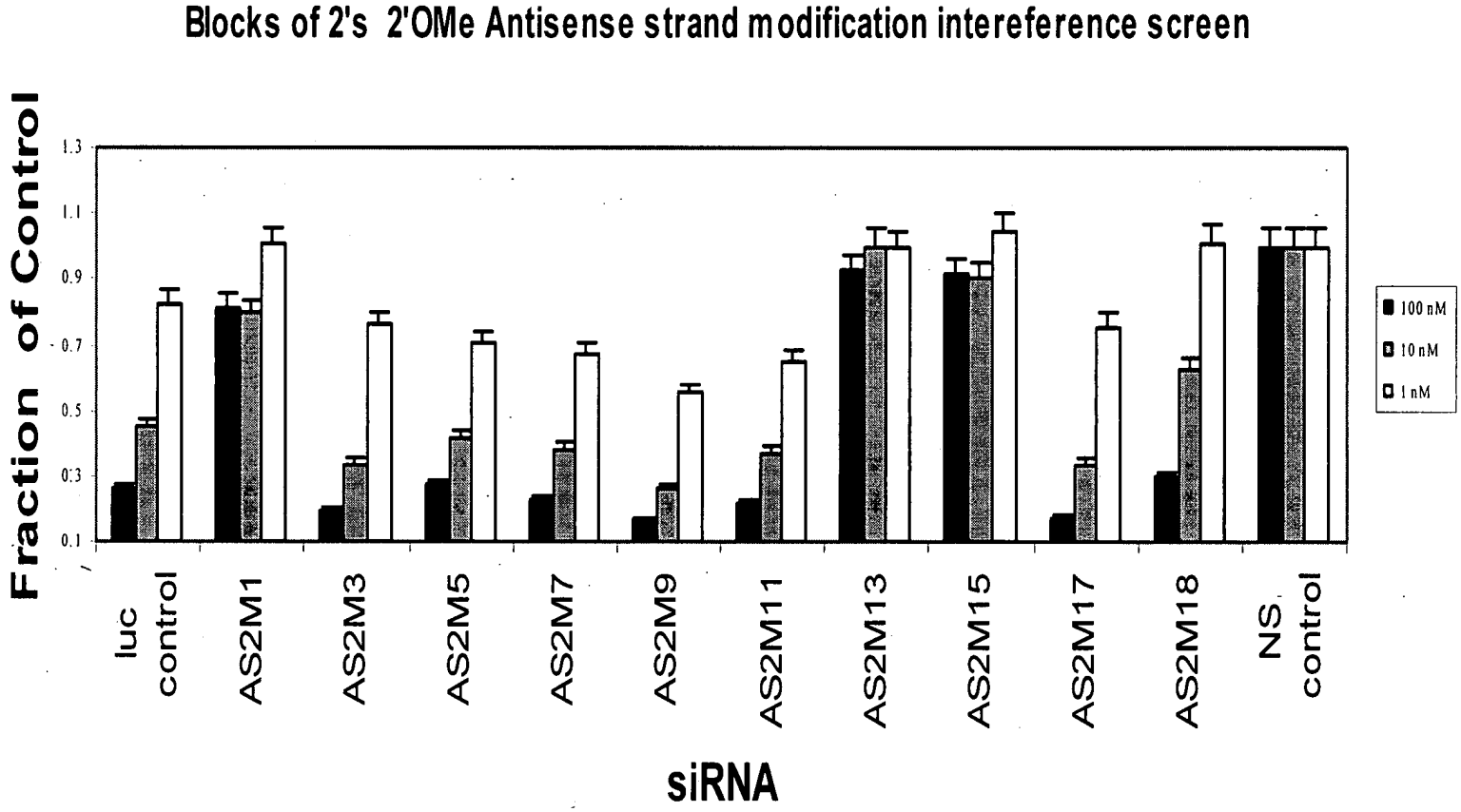


Figure 27: Modification intereference screen: blocks of 2 methoxy in the antisense strand

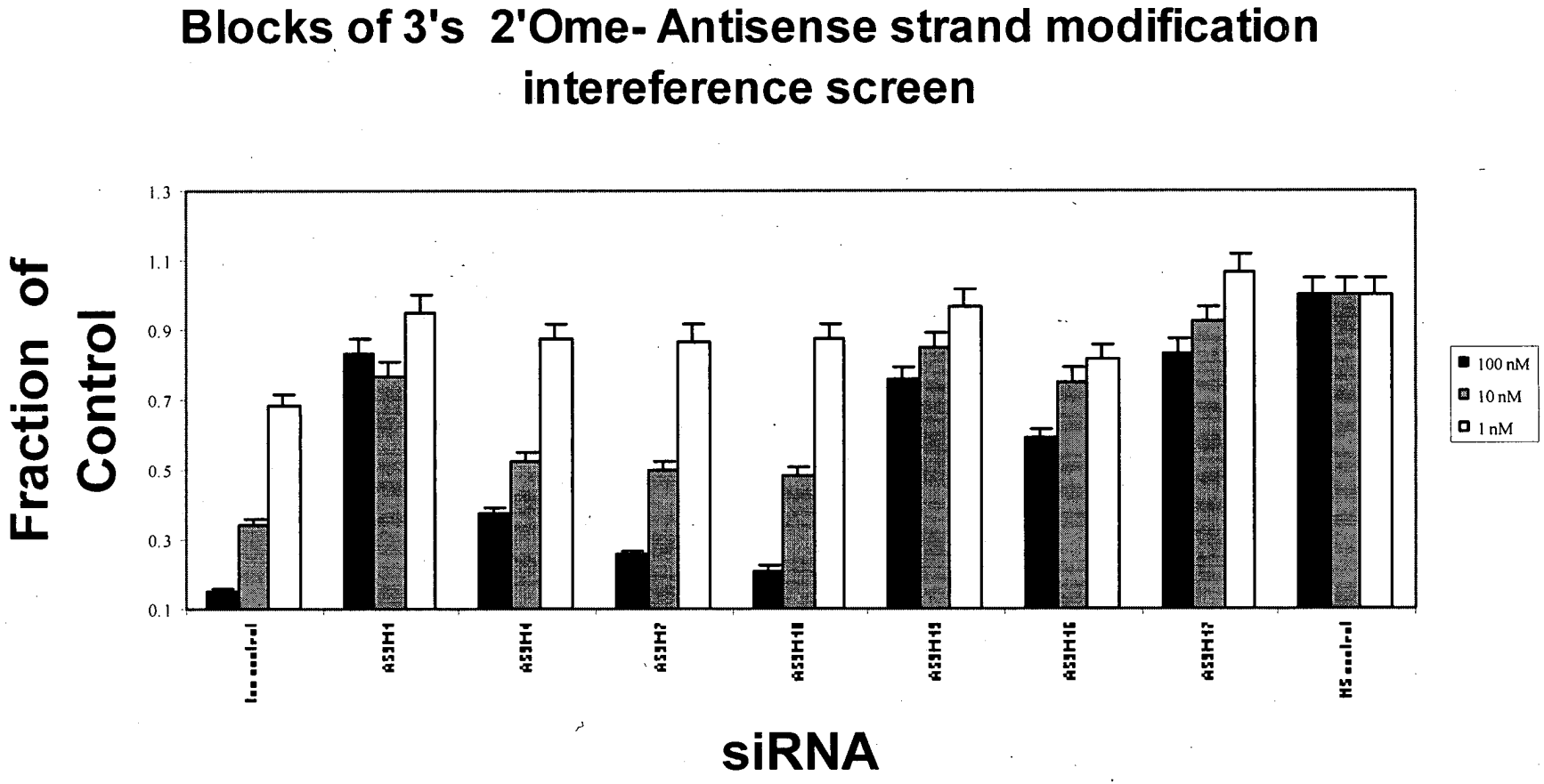


Figure 28: Modification intereference screen: blocks of 3 methoxy in the antisense strand

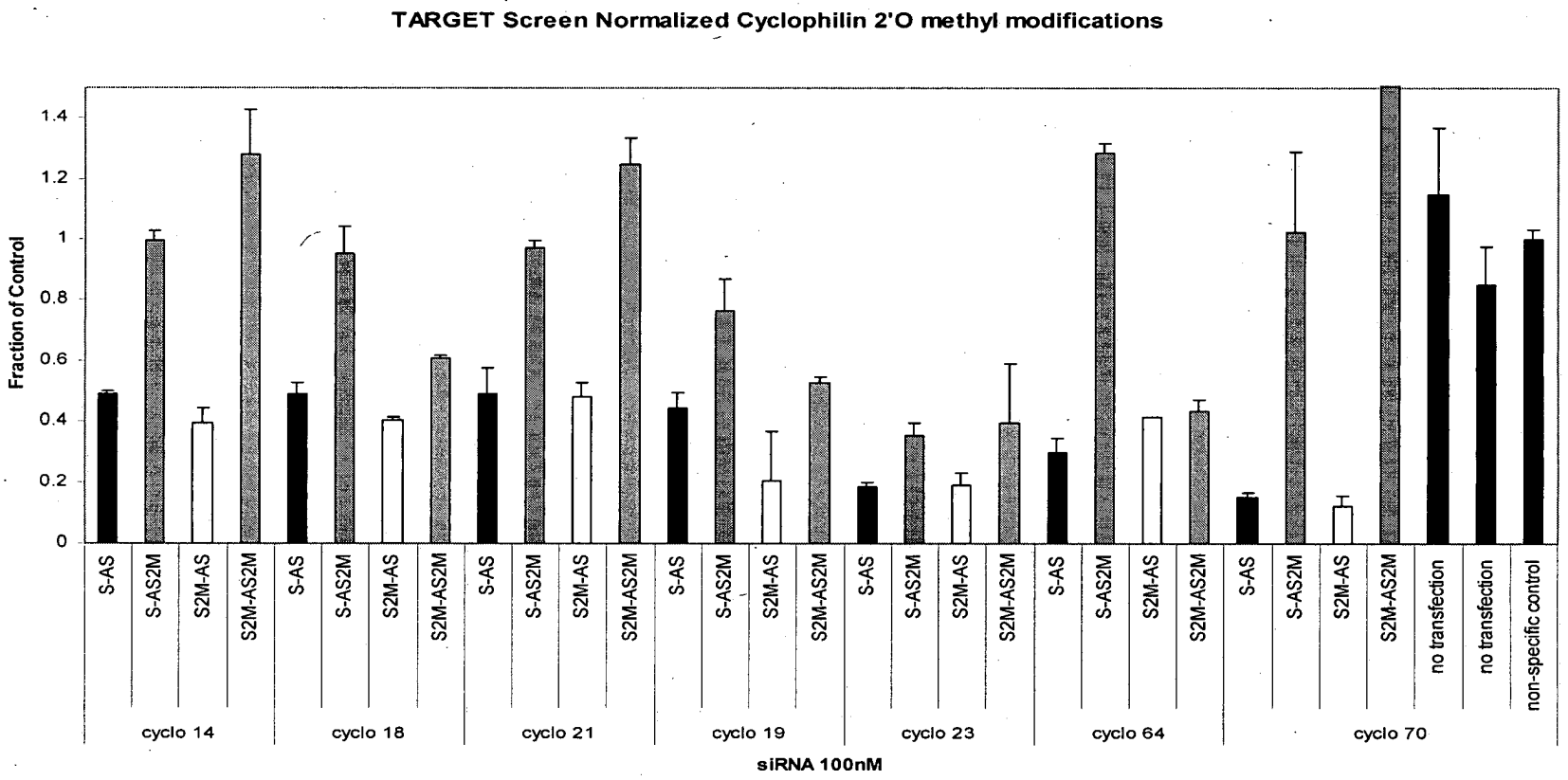


Figure 29: Presence of the 2- 2'Ome modifications result on the 5'AS strand interfere with functionality in human Cyclophilin

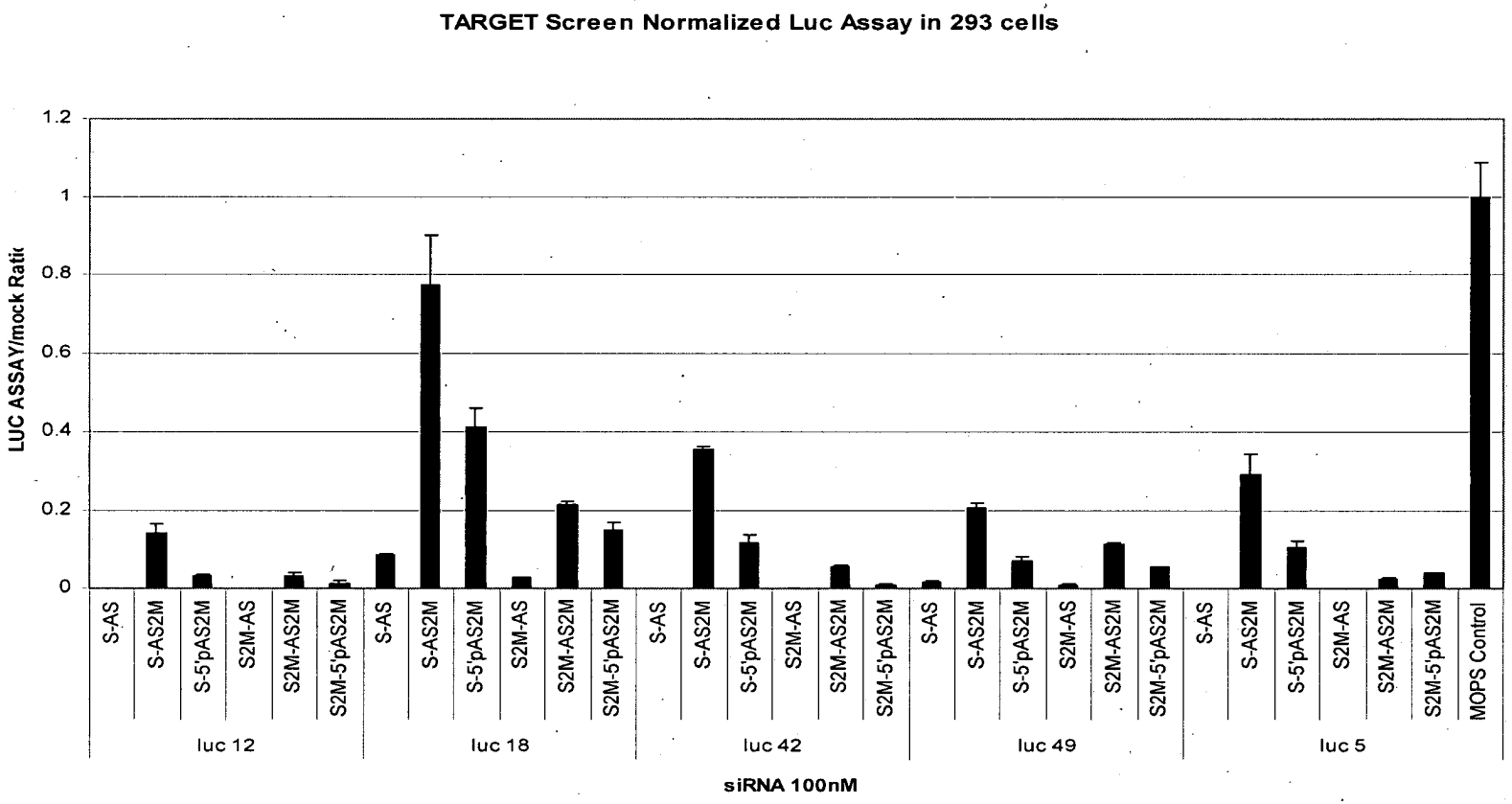


Figure 30: Presence of the 2- 2'Ome modifications result on the 5'AS strand interfere with functionality in the Firefly Luciferase

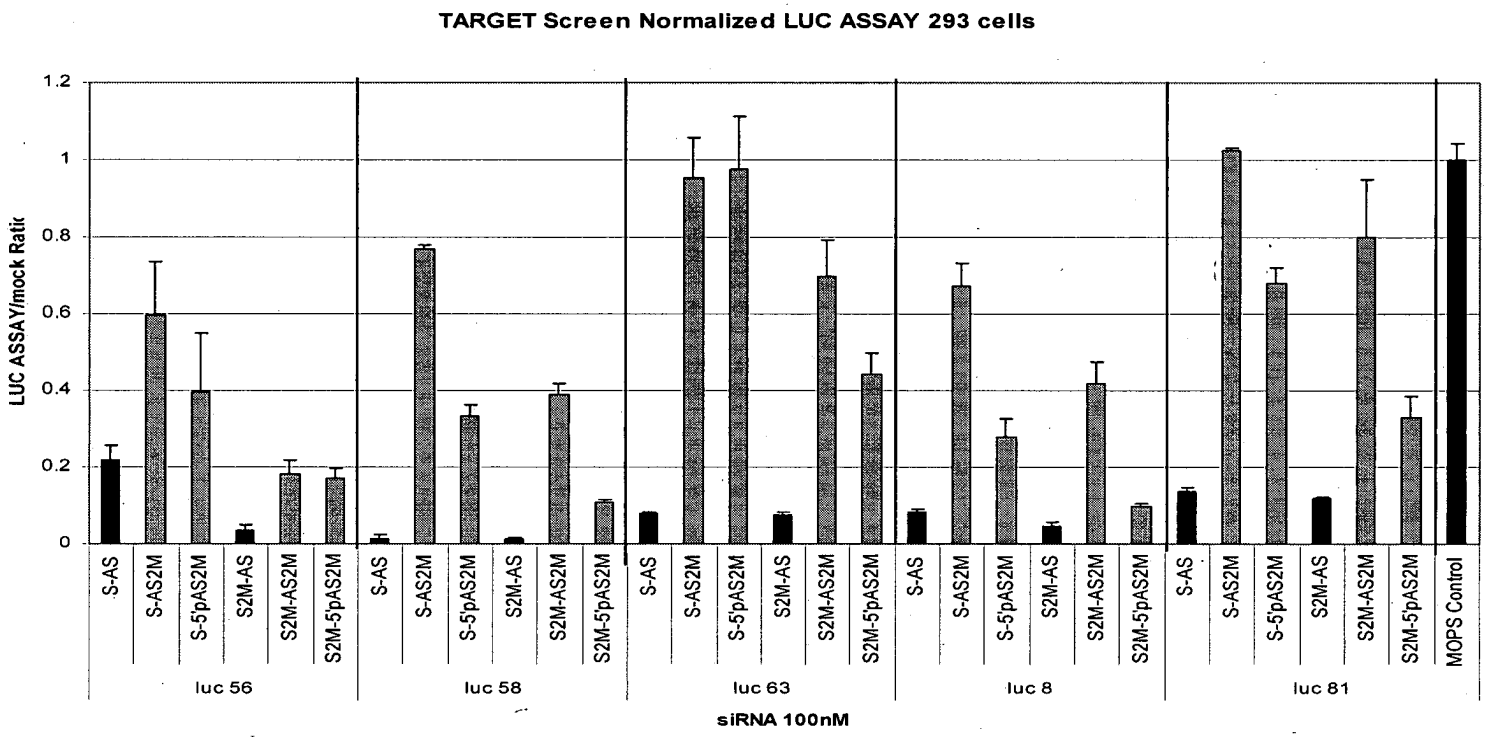


Figure 31: Presence of the 2- 2'Ome modifications result on the 5'AS strand interfere with functionality

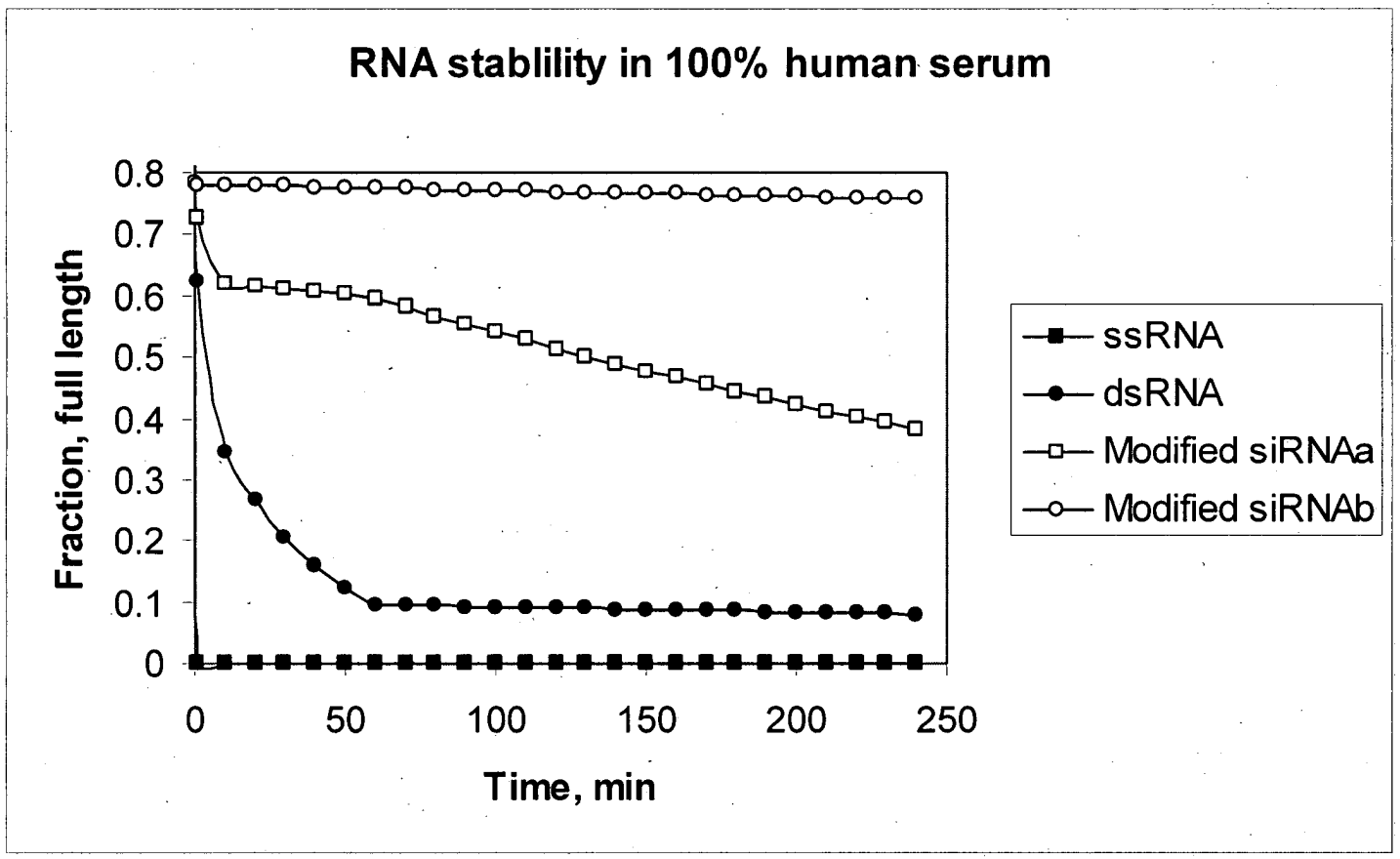


Figure 32: siRNA stability in 100% human serum

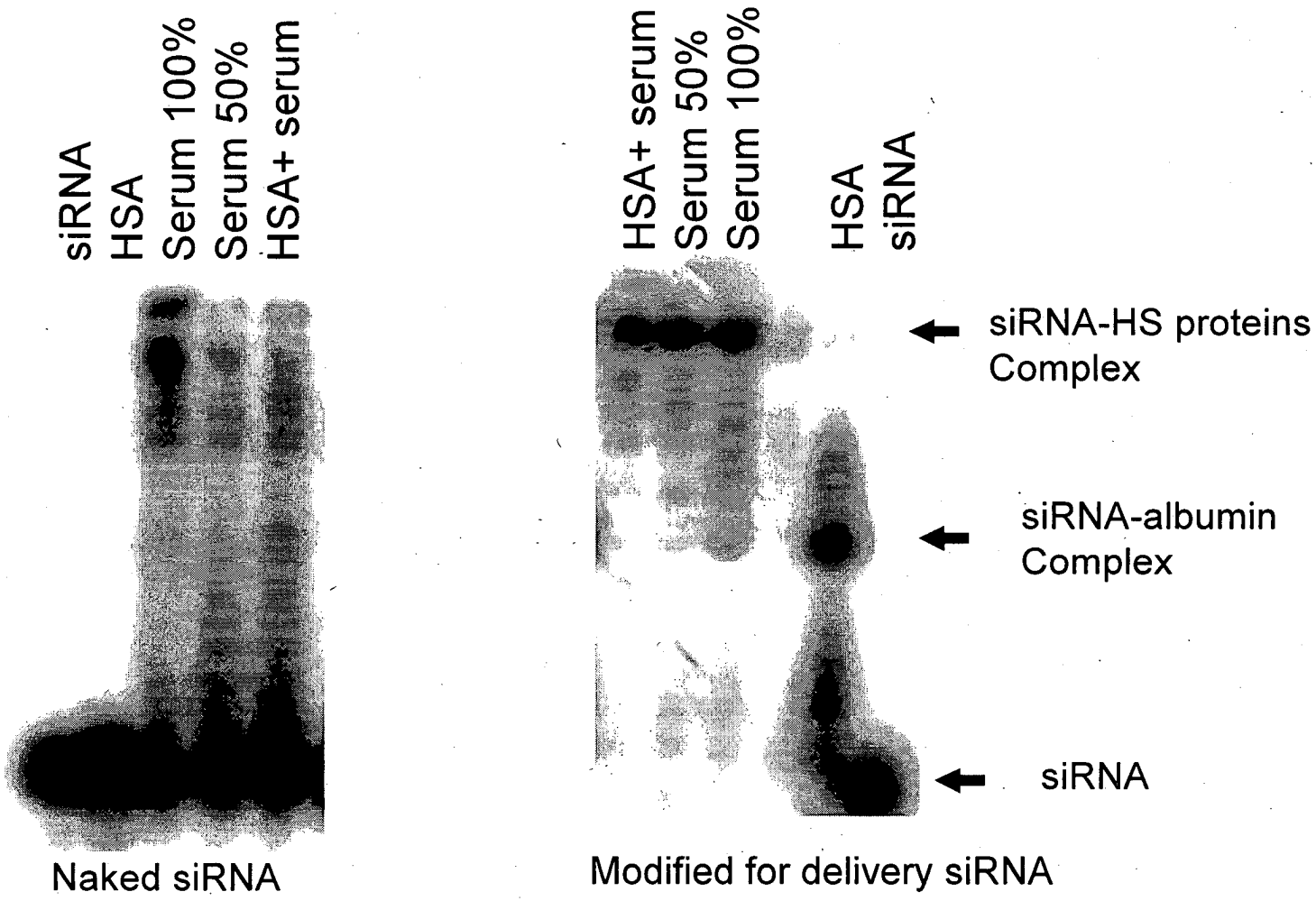


Figure 33: siRNA- cholesterol conjugates has increased affinity to albumin and other serum proteins

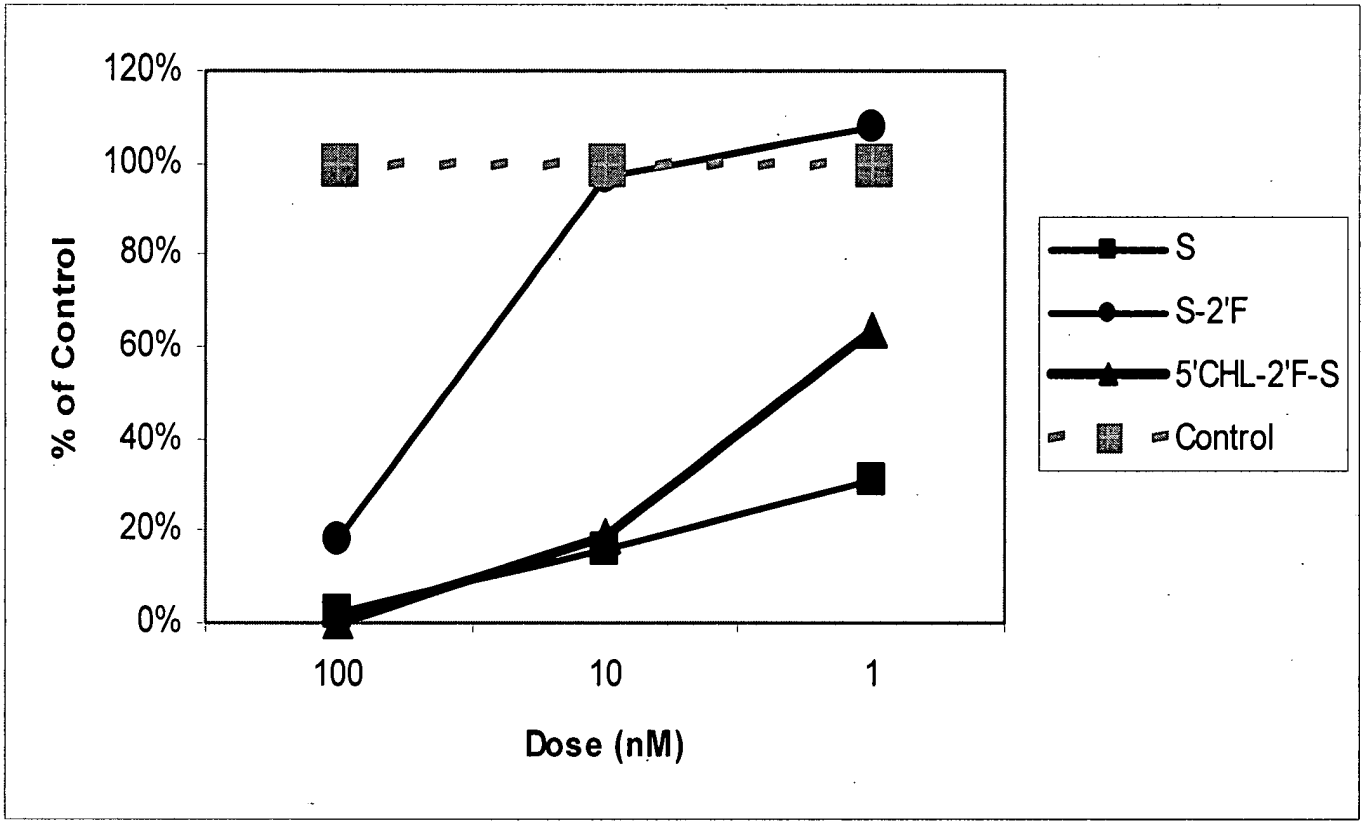


Figure 34: Small Molecule Conjugates Maintain and Accentuate the Potency of Modified siRNA

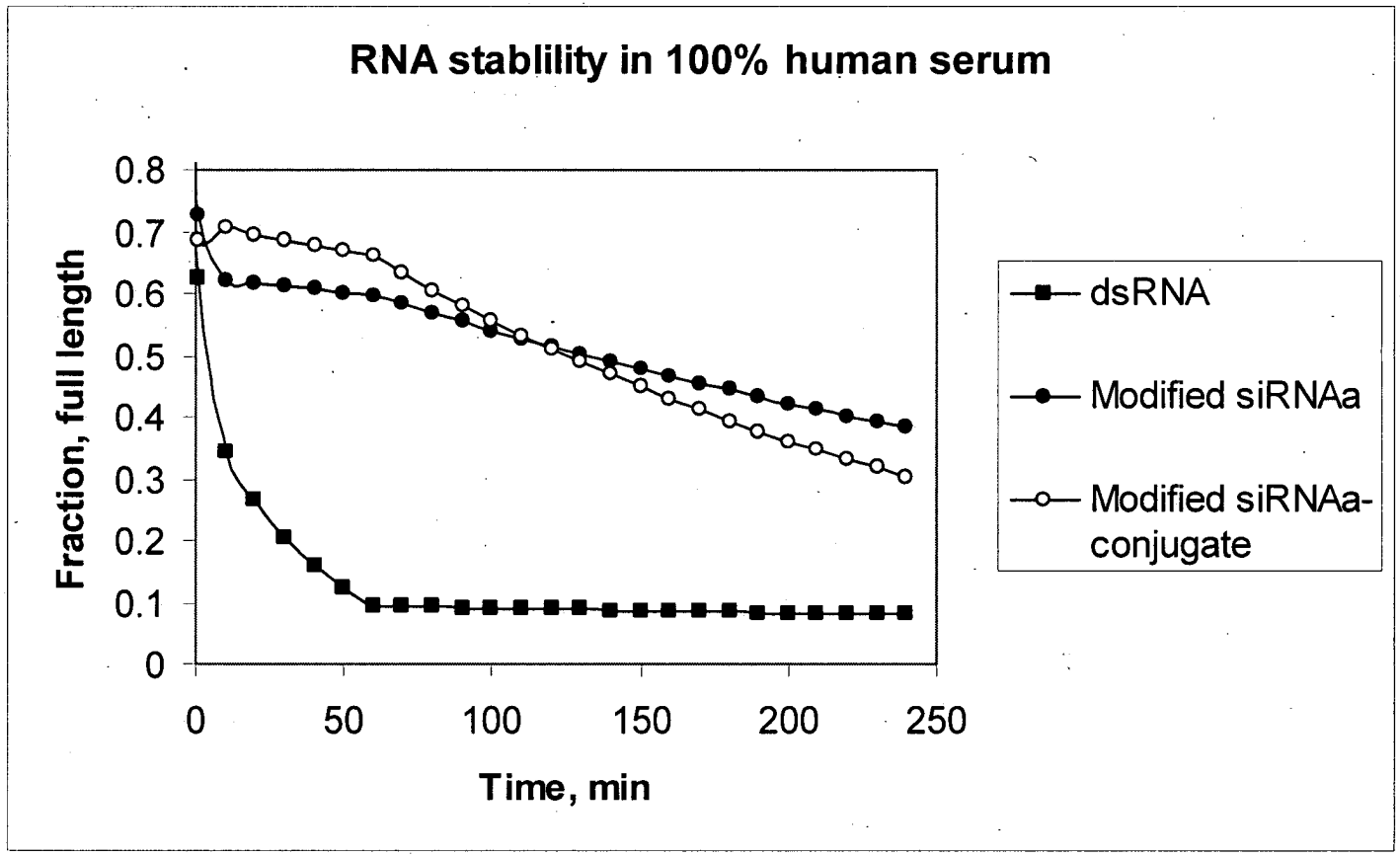


Figure 35: Stability of siRNA conjugates in Human serum

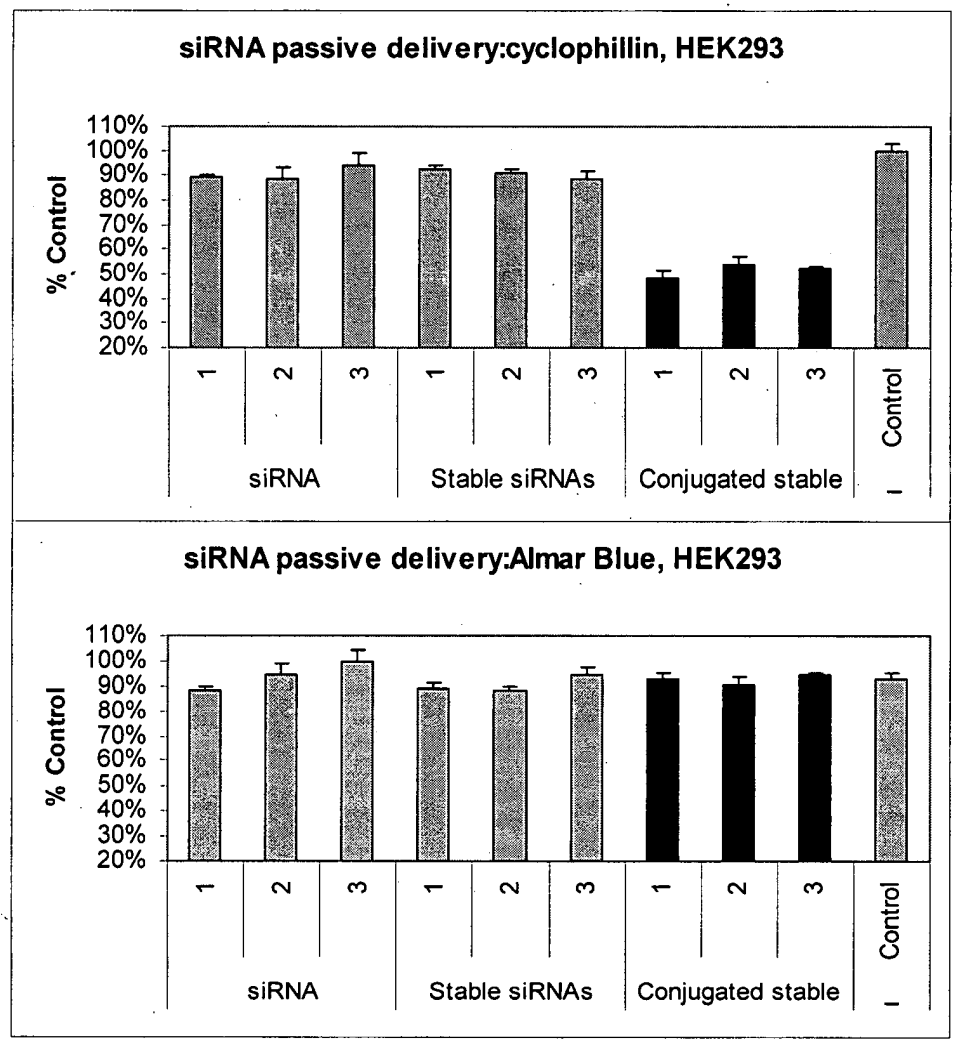


Figure 36: The cholesterol conjugates may induce the siRNA uptake